

Jussi Lampi

# The farm environment, allergic diseases and respiratory health

RESEARCH



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Jussi Lampi

# **The farm environment, allergic diseases and respiratory health**

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Dedicated to Tiina, Oskar, Elias and Mikael

## Abstract

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The farming environment in early childhood may protect from atopy and allergic diseases in childhood and the protective effect may extend into adulthood. On the other hand, among adults farm work is a risk factor for occupational respiratory diseases. The protective effect of the farming environment during early childhood has been hypothesized to be attributable to differences in the quantity and/or quality of microbial exposure. Exposure to environmental microbes may influence the development of the immune system and in that way confers protection from atopy and allergic diseases. However, associations of farming environment during infancy with atopy, allergic diseases and lung function in adulthood have not been investigated in prospective birth cohort studies.

The aim of this thesis was firstly to investigate prospective longitudinal associations of farm-related factors, such as farm animal contact, in infancy with atopy, allergic diseases and lung function at the age of 31 years. Secondly, to investigate the effect of farm-related factors on stimulated cytokine responses among adult women living on farms. Thirdly, to explore whether analysing atopic or non-atopic asthma, as is typically done in epidemiologic studies, can lead to misleading associations.

This thesis utilizes two different study populations. In the Northern Finland Birth Cohort 1966, 6007 subjects born in northern Finland in 1966 were followed up at the age of 31. Being born into a family having farm animals decreased the risk of atopy [adjusted odds ratio (aOR) 0.67; 95% confidence interval (CI) 0.56–0.80], doctor-diagnosed asthma ever (aOR 0.74; 95% CI 0.55–1.00), allergic rhinitis in past 12 months (aOR 0.87; 95% CI 0.73–1.03) and atopic eczema ever (aOR 0.77; 95% CI 0.66–0.91) when the subjects were aged 31. Contact with an increasing number of animal species in childhood was dose-dependently and inversely associated with atopy and allergic diseases in adulthood. Furthermore, to be born into a farming family was associated with higher FEV1 (forced expiratory volume in 1s) and FVC (forced vital capacity) at age 31. No associations with FEV% (FEV1/FVC ratio) were observed. This thesis also illustrates that, in epidemiologic studies, if only data on asthma and atopy are available, the asthma and atopy are best analysed as separate outcomes.

The study population of ALMA2 consisted of adult women, living on farms around the city of Kuopio in eastern Finland. Whole peripheral blood samples were obtained from 112 women. Current or childhood farm related factors displayed only a few associations with stimulated peripheral blood cytokine production (phorbol myristate acetate and ionomycin stimulated interleukine-4, staphylococcal enterotoxin B stimulated interferon- $\gamma$  and lipopolysaccharide stimulated interferon- $\gamma$ ) in these women. Similarly, no strong associations were observed between markers of microbial exposure measured in house dust and cytokine production.

In conclusion, farm animal contact in infancy was associated with a reduced risk of atopy, asthma, allergic rhinitis and atopic eczema at the age of 31. In addition, farming environment in early childhood may have a positive impact on lung function in adulthood. In contrast, the observed associations between the studied farm-related factors and stimulated cytokine production were relatively weak in adulthood. Further studies should be aimed to determine the protective factors beyond farm animal contact and the associated gene-environment interactions.

**Keywords:** Atopy, Asthma, Allergic rhinitis, Farming, Infancy, Cytokines, Lung function



## Tiivistelmä

Jussi Lampi. The farm environment, allergic diseases and respiratory health [Maatilaympäristö, allergiset sairaudet ja hengityselinterveys]. Terveiden ja hyvinvoinnin laitos (THL). Tutkimus 143. 87 sivua. Kuopio, Finland 2014. ISBN 978-952-302-330-7 (painettu); ISBN 978-952-302-331-4 (verkkojulkaisu)

Maatilaympäristö varhaislapsuudessa voi suojata atopiaalta ja allergisilta sairauksilta lapsuudessa ja mahdollisesti myös aikuisiällä. Toisaalta maatilatyö on riskitekijä työperäisille hengityselinsairauksille. Maatilaympäristön suojaavan vaikutuksen on oletettu liittyvän monipuoliseen mikrobialtistukseen, joka saattaa olla yhteydessä immuunijärjestelmän kehittymiseen varhaislapsuudessa ja näin suojata atopiaalta ja allergisilta sairauksilta. Varhaislapsuuden maatilaympäristön yhteyttä atopiaan, allergisiin sairauksiin tai keuhkojen toimintaan aikuisiällä ei kuitenkaan ole tutkittu prospektiivisissa pitkäaikaistutkimuksissa.

Väitöskirjan tavoitteena oli selvittää onko varhaislapsuuden maatilaympäristöön liittyvät tekijät, kuten eläinkontaktit, yhteydessä atopiaan, allergisiin sairauksiin tai keuhkojen toimintaan aikuisiällä. Tavoitteena oli myös tutkia maatilaympäristöön läheisesti liittyvien ympäristötekijöiden yhteyttä maatiloilla asuvien aikuisten naisten stimuloituun sytokiinituotantoon. Tämän lisäksi tavoitteena oli myös tarkastella aiheutuuko epidemiologisissa tutkimuksissa yleisesti käytössä olevista allergisen astman ja ei-allergisen astman määritelmistä vääristyneitä tutkimustuloksia.

Väitöskirja koostuu kahdesta eri tutkimusväestöstä. Pohjois-Suomen syntymäkohortti 1966 -tutkimuksessa 6007 tutkimushenkilöä seurattiin 31 ikävuoteen asti. Tutkimuksessa havaittiin, että syntyminen tuotantoeläimiä kasvattavaan perheeseen oli yhteydessä alentuneeseen atopian riskiin [vakioitu ristitulosuhde (vOR) 0,67; 95 % luottamusväli (LV) 0,56–0,80], elämän aikana lääkärin diagnosoiman astman riskiin (vOR 0,74; 95 % LV 0,55–1,00), viimeisen 12 kuukauden aikana sairastetun allergisen nuhan riskiin (vOR 0,87; 95 % LV 0,73–1,03) ja elämän aikana sairastetun atooppisen ihottuman riskiin (vOR 0,66; 95% LV 0,66–0,91) 31 vuoden ikäisenä. Lapsuusaikaisten eläinlajien määrä perheessä vähensi atopia ja allergisten sairauksien riskiä aikuisiässä annosvasteellisesti. Lisäksi syntyminen maanviljelijäperheeseen oli yhteydessä korkeampaan uloshengityksen sekuntikapasiteettiin ja vitaalikapasiteettiin aikuisiässä. Maatalousympäristö ei kuitenkaan vaikuttanut niiden keskinäiseen suhteeseen. Väitöskirja osoittaa myös, että astma ja atopia tulisi analysoida erillisinä päätetapahtumina epidemiologisissa tutkimuksissa, joissa käytössä on vain tieto astmasta ja atopiasta.

ALMA2-tutkimus koostui aikuisista maataloilla asuvista naisista Kuopion seudulla. Verinäytteet kerättiin 112 tutkimukseen osallistuvalla naisella. Tutkimus osoitti, että vain muutamat nykyiset tai lapsuusajan maatilaympäristöön läheisesti liittyvät tekijät olivat yhteydessä perifeerisen veren stimuloituihin sytokiinipitoisuuksiin (forbolimyristaattiasetaatin ja ionomysiinin yhdistelmällä stimuloitu interleukiini-4, stafylokokki enterotoksiini B:llä stimuloitu interferoni-gamma ja lipopolysakkaridilla stimuloitu interferoni-gamma). Myöskään kotipölynäytteiden mikrobialtistusta kuvaavat merkkiaineet eivät olleet vahvasti yhteydessä sytokiinituotantoon.

Yhteenvedona voidaan todeta, että syntyminen tuotantoeläimiä kasvattavaan perheeseen oli yhteydessä alentuneeseen atopian, astman, allergisen nuhan ja atooppisen ihottuman riskiin 31 vuoden ikäisenä. Tämän lisäksi varhaislapsuuden maatilaympäristöllä voi olla positiivinen vaikutus keuhkofunktion aikuisiällä. Sitä vastoin tutkituilla maatilaympäristöön läheisesti liittyvillä ympäristötekijöillä ei ollut merkittävää yhteyttä stimuloituun sytokiinituotantoon aikuisiällä. Jatkotutkimuksilla voidaan pyrkiä selvittämään tuotantoeläimille altistumiseen liittyviä suojaavia tekijöitä ja mahdollisia geenien ja ympäristön vuorovaikutuksia.

Avainsanat: atopia, astma, allerginen nuha, maanviljelys, varhaislapsuus, sytokiinit, keuhkofunktio.

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## List of original papers

- I      Lampi J, Canoy D, Jarvis D, Hartikainen AL, Keski-Nisula L, Järvelin MR, Pekkanen J. Farming environment and prevalence of atopy at age 31: prospective birth cohort study in Finland. *Clin Exp Allergy*. 2011 Jul;41(7):987-93
- II     Lampi J, Koskela H, Hartikainen AL, Ramasamy A, Alves AC, Järvelin MR, Pekkanen J. Farm environment during infancy and lung function at age 31 – Prospective birth cohort study in Finland. Submitted.
- III    Lampi J, Roponen M, Hyvärinen A, Hirvonen MR, Larsson L, Nevalainen A, Pekkanen J. Determinants of stimulated peripheral blood cytokine production among farming women. *Int J Hyg Environ Health*. 2011 Jun;214(3):205-9.
- IV    Pekkanen J, Lampi J, Genuneit J, Hartikainen AL, Järvelin MR. Analyzing atopic and non-atopic asthma. *Eur J Epidemiol* 2012 Apr;27(4):281-6

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## Abbreviations

aOR	Adjusted odds ratio
DD	Doctor-diagnosed
FEV1	Forced expiratory volume in one second
FEV%	FEV1/FVC ratio
FVC	Forced vital capacity
IFN- $\gamma$	Interferon-gamma
IgE	Immunoglobulin-E
IL	Interleukin
LPS	Lipopolysaccharide
OR	Odds ratio
P/I	Phorbol myristate acetate and ionomycin
RR	Risk ratio
SEB	Staphylococcal enterotoxin B
SPT	Skin prick test
SR	Self-reported
TNF- $\alpha$	Tumor necrosis factor-alpha
TNF- $\beta$	Tumor necrosis factor-beta
T <sub>h</sub> 1/ T <sub>h</sub> 2	T-helper cell subsets 1 and 2

# 1 Introduction

Allergic diseases, such as asthma, allergic rhinitis and atopic eczema, are a major public health issue in western countries and the prevalence of asthma and atopy has been increasing in last decades (Eder et al. 2006). In the 1989 so called hygiene hypothesis was developed. The initial hypothesis was that declining family size and improved hygiene could be risk factors for atopic diseases as those may reduce the risk of infections in early childhood (Strachan 1989). This hypothesis offered an explanation for the epidemic of allergic diseases occurring in western countries. Since then, the focus has shifted to environmental factors during early childhood, such as environmental microbial exposure, as possible preventive factors against allergic diseases.

The possibility that living in a farming environment during early childhood is associated with a decreased risk of atopy and allergic diseases during childhood and that the effect may continue to adulthood is comprehensively reviewed in this thesis. Complex biological mechanisms behind the preventive effect of farming environment are hypothesized to be attributable to differences in the quantity and/or quality of microbial exposure (von Mutius et al. 2000; Braun-Fahrlander et al. 2002; Schram et al. 2005; Ege et al. 2011). Exposure to environmental microbes, such as endotoxin which is a cell wall component of gram-negative bacteria, may affect the development of the immune system (Braun-Fahrlander et al. 2002; Roponen et al. 2005) and thereby protect from atopy and allergic diseases (Braun-Fahrlander et al. 2002; Gehring et al. 2002; Ege et al. 2007; Ege et al. 2011). On the other hand, among adults farm work is a risk factor for occupational respiratory diseases (Linaker et al. 2002).

Farming environment may also regulate the production of the receptors of innate immunity especially pattern recognition receptors. Pattern recognition receptors, such as CD14 and Toll-like receptors, may mediate the effect of microbial exposure and the development of the immune system (von Mutius et al. 2010). Gene expression of pattern recognition receptors has been reported to be associated with the farming environment (Lauener et al. 2002; Ege et al. 2006).

The preventive effect of farming environment on atopy and allergic diseases may also result from gene-environment interactions. Single nucleotide polymorphism in genes coding pattern recognition receptors of the innate immunity, such as CD14 and toll-like receptors, may modify the associations of farm environment with atopy and asthma (Leynaert et al., 2006; Smit et al., 2009). Therefore, the development of atopy and allergic diseases may be a combination

of environmental and genetic factors during immune system development (Leynaert et al., 2006; Smit et al., 2009).



# 2 Review of the literature

## 2.1 The definitions of atopy and allergic diseases

### 2.1.1 Atopy

In the report of the nomenclature review committee of the World Allergy Organization atopy is defined as “a personal and/or familial tendency, usually in childhood or adolescence, to become sensitized and produce Immunoglobulin-E (IgE) antibodies in response to ordinary exposures to allergens, usually proteins. As a consequence, these persons can develop typical symptoms of asthma, rhinoconjunctivitis, or eczema” (Johansson et al. 2004). In the epidemiologic studies, reviewed in this thesis, atopy is defined as skin prick test positivity (skin reaction is used to evaluate IgE sensitivity for tested allergen) or specific serum IgE-antibodies against any of tested allergens (tables 1, 2, 5 and 8).

### 2.1.2 Asthma

In the Global Initiative for Asthma report (GINA, 2012), asthma is defined as follows. “Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment”. Defining asthma in epidemiologic studies is difficult and there are several definitions currently in use (Sa-Sousa et al. 2014). In the epidemiologic studies reviewed in this thesis, the main focus has been on reported doctor-diagnosed asthma, which is recommended in studies focusing on risk factors of asthma (Pekkanen et al. 1999). However, if this strict definition was not reported, then a secondary definition has been utilized e.g. self-reported asthma or self-reported symptoms of asthma (table 3, 4, 6, 7 and 9).

#### 2.1.2.1 Analysing atopic and non-atopic asthma in epidemiologic studies

Allergens are major triggers of asthma attacks and being atopic increases the risk of persistent asthma (Vonk et al. 2006; Sears et al. 2003). For example, Sears et al. (2003) reported in a longitudinal study that house dust mite sensitization was associated with the risk of persistent asthma (OR 2.41; 95% CI 1.42-4.09). This has led to the old distinction between atopic and non-atopic asthma. The definition of atopic asthma is typically based on a positive skin prick test or specific IgE

antibodies against common allergens. This kind of definition of atopic and non-atopic asthma is currently widely used when designing and analysing studies on the determinants and characteristics of asthma (e.g. Rönmark et al. 1999; Moncayo et al. 2010). However, only little more than half of asthma is attributable to atopy in the developed world (Pearce et al. 1999) and much less in the developing world (Moncayo et al. 2010). In addition, the prevalence of atopy among asthmatics is mainly determined by the general prevalence of atopy in the population (Ronchetti et al. 2009). Therefore, the finding that an asthmatic child is atopic “does not necessarily mean that the disease is allergic in nature or that it is causing asthma” (GINA, 2012). Nonetheless the great interest of defining phenotypes and endotypes of asthma (Lötvall et al. 2011; Wenzel, 2006), this definition of atopic and non-atopic asthma continues to be used and will probably be used also in the future, as many large epidemiological studies have data only on diagnoses of asthma and atopy.

### 2.1.3 Allergic rhinitis and rhinoconjunctivitis

In the Allergic Rhinitis and Its Impact on Asthma report (ARIA, 2008), Rhinitis is defined as “an inflammation of the lining of the nose and is characterized by nasal symptoms including anterior or posterior rhinorrhoea, sneezing, nasal blockage and/or itching of the nose” and furthermore allergic rhinitis is defined as “Allergic rhinitis is associated with an IgE-mediated immune response against allergens. It is often associated with ocular symptoms”. Furthermore, the report of the nomenclature review committee of the World Allergy Organization defines allergic rhinoconjunctivitis as “IgE-mediated allergic conjunctivitis commonly accompanies allergic rhinitis, so this disorder is appropriately termed allergic rhinoconjunctivitis” (Johansson et al. 2004). In the epidemiologic studies reviewed in this thesis, the main focus has been on reported doctor-diagnosed hay fever or allergic rhinitis/rhinoconjunctivitis. This definition is generally used in epidemiologic studies but it may underestimate the actual prevalence allergic rhinitis (Skoner 2001). However, if such definitions were not reported, secondary definitions could be either self-reported or self-reported symptoms of hay fever or allergic rhinitis/rhinoconjunctivitis (table 3, 4, 6, 7 and 9).

### 2.1.4 Atopic eczema

In the European Academy of Dermatology and Venereology eczema task force position paper atopic eczema is defined as “an inflammatory, chronically relapsing and intensely pruritic skin disease occurring often in families with atopic diseases (atopic dermatitis, bronchial asthma and/or allergic rhino-conjunctivitis). Eczema is a non-contagious inflammation of epidermis and dermis with characteristic clinical (itch, erythema, papule, seropapule, vesicle, squames, crusts, lichenification, in synchronous or metachronous polymorphy) and

dermatopathological (spongiosis, acanthosis, hyper- and parakeratosis, lymphocytic infiltrates and exocytosis, eosinophils) signs’’ (Darsow et al. 2009). However, diagnosis of atopic eczema is clinical and there are several diagnostic criteria available (Eichenfield et al. 2014). In the epidemiologic studies reviewed in this thesis, the main focus, if otherwise not stated, has been on reported doctor-diagnosed atopic eczema. Doctor-diagnosed atopic eczema may underestimate the prevalence of atopic eczema. However, it gives better accuracy compared to questionnaire based on self-reported symptoms (Flohr et al. 2009). If this definition was not reported, secondary definitions could be either self-reported atopic eczema or self-reported symptoms of atopic eczema, respectively (table 3, 4, 6, 7 and 9).

## **2.2 Living on a farm in early childhood, atopy and allergic diseases**

### **2.2.1 Atopy**

In 1999, the cross-sectional study conducted by Braun-Fahrlander et al. (1999) found an association between parental farming and decreased risk of atopy during childhood. Since then, numerous studies investigating the associations between farm environment during early childhood and atopy have been published. Tables 1 and 2 summarize the reports examining associations between farming environment during early childhood and atopy in childhood and adulthood, respectively. The majority i.e. 14 of 23 studies listed in table 1, detected statistically significant inverse associations between farm environment and atopy during childhood (i.e. a protective effect). The studies have mainly been cross-sectional in their design.

From the very onset, attempts have been made to investigate the farm in greater detail. Riedler et al reported that the farm environment could protect from atopy during childhood and that this association may be due to contact with farm animals (Riedler et al. 2000). In a study conducted in two different regions, associations between living in a farm and atopy in childhood varied between the regions with differences noted in the type of farming being practiced (livestock vs. crop farming) (Downs et al. 2001). Barnes et al. (2001) reported that the farming environment could protect from atopy during childhood. However, the association disappeared when rural and urban children were analysed separately (Barnes et al. 2001). Thus, the protective effect does not seem to be associated simply to the farming environment but more to lifestyle and environmental features which are different from the urban environment.

There have been a few longitudinal studies published on this topic and as far as I am aware, only one prospective longitudinal study has been published (Depner et

al. 2013). In this prospective birth cohort study, farming was not associated with the incidence of sensitization to seasonal allergens at the age of one year (Depner et al. 2013). Horak et al. (2002) detected a significant inverse association between parental farming and the incidence of atopy in childhood. In the same study, subjects with parental farming were more likely to lose their skin prick test positivity during the follow-up period. However, there was no information about farming status during early childhood since the farming status was defined from the questionnaire taken during the follow-up study (Horak et al. 2002).

**Table 1.** Summary of studies investigating associations between living in a farm environment and atopy as assessed with skin prick tests (SPT) or serum IgE-antibodies (IgE) for specific allergens in childhood.

Ref.	Year	N	Age#	Method	Atopy $\alpha$
<i>Health assessment in childhood or adolescence</i>					
Braun-Fahrlander et al.	1999	404	13-15	IgE	↓↓
Ernst et al.	2000	1199	12-19	SPT	↓↓
Riedler et al.	2000	1137	8-10	SPT	↓↓
Riedler et al.	2001	901	6-13	IgE	↓↓
Klintberg et al.	2001	650	7-8	SPT	↓
Downs et al.	2001	1500	7-12	SPT	↓
Barnes et al.	2001	929	11-19	SPT	↓↓
Braun-Fahrlander et al.	2002	812	6-13	IgE	↓↓
Wickens et al.	2002	275	7-10	SPT	↑
Horak et al.	2002	844	11**	SPT	↓↓i
Remes et al.	2003;2005	710	6-13	SPT	↓↓
Merchant et al.	2005	351/215	8-18	IgE/SPT	↓/↓
Ege et al.	2006	2086	5-13	IgE	↓↓
Alvén et al.	2006	4049	5-13	IgE	↓↓
von Hertzen et al.	2006	813	7-16	SPT	↓↓
Zekveld et al.	2006	797	7-18	SPT	n.s.
Perkin et al.	2006	879	children	SPT	↓
Ege et al.	2011	6843 / 9668	6-13 / 6-12	IgE	↓↓
Lee et al.	2012	1507	9 - 12	SPT	↓
Macneill et al.	2013	2586	9**	IgE/SPT	↓↓/↓
Genuneit et al.; Fuchs et al.	2013; 2012	8023	6-12	IgE	↓↓
Depner et al. (P)	2013	793	1	IgE	↑i
Cooper et al.	2013	6821	5-16	SPT	↑

\* Living in any farm, to be born in any farm or parental farming. For studies examining at farms with farm animals in more detail see table 5.

#Age of assessment of allergic sensitization; \*\*Mean age;  $\alpha$ Sensitization to any allergen studied if otherwise not mentioned

↓↓ Statistically significant inverse association ↑↑ Statistically significant positive association; i: Incidence

↓ or ↑ Direction of statistically non-significant association, regardless of effect size

n.s Statistically not significant association, direction of the effect not reported. (P) Prospective longitudinal study

The protective effect of the farm environment during early childhood seems to continue also into adulthood. In all, 6 of 8 studies in table 2 reported statistically significant inverse associations between farm environment during early childhood and atopy during adulthood (i.e. a protective effect). Only one prospective longitudinal study has been published to date (Pekkanen et al. 2001). In this large prospective cohort study there was an inverse association between paternal

farming during infancy and decreased risk of atopy in adulthood. However, in that study the information about the childhood farm environment was limited to paternal farming (Pekkanen et al. 2001).

In a cohort study, Portengen et al. (2002) reported that living on a farm during childhood was associated with a decreased risk of atopy during adulthood independently of current farming status. Similarly, having a farm childhood has been reported to protect from atopy in adulthood also in a few other cross-sectional studies (table 2). These studies indicate that the farming environment during childhood may confer life-long protection against atopy. Kilpeläinen et al. (2002) investigated associations between the farm environment during childhood and specific sensitization to individual allergens at adult age. The farming environment was significantly associated with an increased risk of house-dust mite sensitization and a decreased risk of cat sensitization. Therefore, the protective effect might not be related to all allergens. (Kilpeläinen et al. 2002).

There is evidence from cross-sectional studies that the farm environment may protect from atopy also prenatally. In the cross-sectional study of Riedler et al. (2001) it was reported that children whose mothers were active on the farms during pregnancy had a lower risk of atopy compared to children with non-active mothers. Ege et al. (2006) reported that prenatal exposure to the stables could protect from atopy in childhood independently from more current farm-related factors. However, in a recent cross-sectional study, no association was detected between prenatal farm animal contact and atopy in childhood (Lee et al. 2012). In a prospective birth cohort study conducted in five European countries, maternal exposures to animal barns during pregnancy were associated with a decreased risk of having IgE-antibodies for seasonal allergens in cord blood (Ege et al. 2008).

The above mentioned studies indicate that the protective effect may occur even during fetal development. However, it is difficult to assess the effect of the timing, because the exposure is likely to remain similar both during the pregnancy and after the delivery among women living on farms. In the study of Ege et al. (2006) 20% of pregnant mothers had stopped working in stables. The protective effect was somewhat weaker after the delivery and was no longer statistically significant after further adjustment for doing farm work during pregnancy. This emphasizes the importance of the maternal exposure to the farming environment during the pregnancy (Ege et al. 2006).

In conclusion, the majority of studies published have found that the farm environment during early childhood protects from atopy in childhood and also in adulthood. This is in line with the meta-analysis published by Perkin et al. (2006)

which reported that pooled estimated for atopy in childhood was 0.59 (95% CI 0.52-0.68). The reviewed studies suggest that the protective effect may even occur at the time of fetal development. However, there is a lack of prospective longitudinal studies.

**Table 2.** Summary of studies investigating associations between living in a farm environment during childhood and atopy as assessed with skin prick tests (SPT) or serum IgE-antibodies (IgE) for specific allergens in adulthood.

Ref.	Year	N	Age#	Method	Atopy <sup>a</sup>
Health assessment in adulthood					
Leynaert et al.	2001	6251	20-44	IgE	↓↓
Pekkanen et al. (P)	2001	5192	31	SPT	↓↓
Portengen et al.	2002	1387/439	19-20**	SPT/IgE	↓↓/↓
Kauffman et al.	2002	805	43**	SPT	↓
Koskela et al.	2005	433	39/42**	SPT	↓
Radon et al.	2004	321	18-44	IgE	↓↓
Leynaert et al.	2006	600	20-44	IgE	↓↓
Schulze et al.	2007	1595	18-44	IgE	↓↓

\* Living in any farm, to be born in any farm or parental farming. For studies examining at farms with farm animals in more detail see table 5.

#Age of assessment of atopy; \*\*Mean age; <sup>a</sup>Sensitization to any allergen studied if otherwise not mentioned

↓↓ Statistically significant inverse association ↑↑ Statistically significant positive association

↓ or ↑ Direction of statistically non-significant association, regardless of effect size

n.s Statistically not significant association, direction of the effect not reported. (P) Prospective longitudinal study

## 2.2.2 Asthma

The farm environment during early childhood may also prevent from the clinical manifestation of allergic diseases. Tables 3 and 4 summarize the studies which have investigated the associations between the farming environment in childhood and asthma in childhood and adulthood, respectively. The majority, 16 of 26, of the described studies in table 3 reported statistically significant inverse association between farm environment during early childhood and asthma in childhood (i.e. a protective effect). However, the studies have mainly been cross-sectional in their design. It seems that there are only two prospective longitudinal studies. In the longitudinal prospective study by Kiechl-Kohlendorfer et al. (2007) a significant inverse association was detected between farming environment and atopic asthma in childhood. Similar longitudinal association with incidence of asthma was found in another recent study (Midodzi et al. 2007). Furthermore, a recent meta-analysis described a 25% lower prevalence of asthma among subjects with a childhood farming environment (Genuneit, 2012) which was in line with the values from the meta-analysis conducted by Perkin et al (2006) which reported that the pooled estimate for asthma was 0.71 (95% CI 0.64-0.80).

The preventive effect of childhood farm environment on asthma seems to continue into the adulthood with 8 of 16 studies in table 4 reporting significant

inverse associations between farming environment during early childhood and asthma during adulthood (i.e. a protective effect). There appears to be only one prospective longitudinal study reporting an inverse association between paternal farming in early childhood and asthma in adulthood. However, in that study, the information about the exposure to the farm environment during infancy was limited to paternal farming (Pekkanen et al. 2001).

In a cohort study conducted in France, farming environment during childhood reduced the risk of both childhood and adult onset asthma in females (Varraso et al. 2012). In a large register cohort, Braback et al. (2004) detected significant inverse associations between the childhood farm environment and asthma in adulthood. However, this association was only seen in the cohort 1972-1981 but not in earlier cohorts. The longitudinal study of Omland et al. (2011) described an inverse association between the childhood farming environment and the incidence of asthma in adulthood. However, current exposure to the farm animals seemed to increase the risk of new asthma (Omland et al. 2011). In all of above mentioned cohort studies, information about childhood farming environment was collected retrospectively.

Some studies have investigated the effect of prenatal exposure to the farming environment on the risk of asthma. Ege et al. (2006) reported that mother's exposure to the stables during pregnancy was not statistically associated with asthma their children. A similar association has been reported in other recent cross-sectional studies (Lee et al. 2012). In their cross-sectional study, Douwes et al. (2008) reported that prenatal farm animal exposure could protect from asthma in childhood. However, combination of prenatal and current exposure was most protective against asthma in the study (Douwes et al., 2008).

In conclusion, the farm environment during early childhood may protect from the development of asthma in childhood and the preventive effect seems to continue to the adulthood. However, there is a lack of longitudinal prospective studies examining this topic. The effect of prenatal exposure to the farming environment during pregnancy has remained unclear.

**Table 3.** Summary of studies investigating associations between living in a farm environment\* and allergic disease (ever during lifetime if otherwise not mentioned) in childhood.

Ref.	Year	N	Age#	Asthma		AR/HF		AE	
				Def.	Results	Def.	Results	Def.	Results
Health assessment in childhood or adolescence									
Braun-Fahrlander et al.	1999	1620	6-15	SR	↑	HF;SR	↓	SRS	↓
von Ehrenstein et al.	2000	10163	5-7	DD	↓	HF;DD	↓↓	DD	↑
Riedler et al.	2000	2283	8-10	DD	↓↓	HF;DD	↓↓	SRS	↓
Ernst et al.	2000	1199	12-19	SRS12+LF	↓↓	----	----	----	----
Riedler et al.	2001	2618	6-13	SRS12	↓↓	SRS12	↓↓	SRS	n.s.
Klinberg et al.	2001	707	7-8	SR	↓↓	HF;SR	↓↓	SRS	↓
Downs et al.	2001	1500	7-12	DD	↓	HF;SR12	↓	----	----
Braun-Fahrlander et al.	2002	812	6-13	DD;AA	↓↓	HF;DD	↓↓	----	----
Wickens et al	2002	293	7-10	SR	↓	HF;SR	↑	SR	↓
Remes et al.	2002	7981	13-14	DD	↑	HF;SR	↓	SR	↑
Chrischilles et al	2004	3090	6-14	DD	↓	----	----	----	----
Benn et al. (P)	2004	24341	1.5	----	↓	----	----	SRS;i	↓
Merchant et al.	2005	644	1-17	DD	↑	----	----	----	----
Adler et al.	2005	4152	10 <sup>a</sup>	DD	↓↓	HF; SR	↓↓	SR;e	ns.
Brunner et al.	2005	13490	13-21	DD	↓↓	----	----	----	----
Remes et al.	2005	710	6-13	DD	↓	HF;DD	↓	DD	↑
Ege et al.	2006	8263	5-13	DD	↓	HF; DD	↓↓	----	----
Dimich-ward et al.	2006	1158	8-20	DD	↓↓	AR;SRS12	↓↓	SRS12	↓↓
Alvfen et al	2006	14893	5-13	DD	↓↓	HF; DD	↓↓	DD	↓
Perkin et al.	2006	4767	childhood	SRS12	↓↓	AR;SRS12	↓↓	SRS12	↓
Mlodzli et al. (P)	2007	13524	2-13	DD; i	↓↓	----	----	----	----
Kiechl-Kohlendorfer et al. (P)	2007	38808	6-10	DD;AA	↓↓	----	----	----	----
Douwes et al.	2008	1899	5-17	SR	↓↓	HF;SR	↓	SR; e	↓↓
Matheson et al. (P)	2009	3429	7	----	----	HF; SRS	↓	----	----
Ege et al.	2011	6843 / 9668	6-13 / 6-12	DD	↓↓	----	----	----	----
Roduit et al. (P)	2011	1063	1-2	----	----	----	----	DD;i	↓
Matheson et al.	2011	8486	11-20	----	----	HF; SR;i	↓↓	----	----
Lee et al.	2012	1749	9-12	DD	↓	AR;DD	↓↓	DD	↓
Varraso et al.	2012	54018	16	DD	↓↓	----	----	----	----
Barry et al.	2014	2259	6-18	DD	↓	----	----	----	----
Cooper et al.	2013	6821	5-16	----	↓	AR; SRS	↓	SRS	↑

\* Living in any farm, to be born in any farm or parental farming. For studies examining at farms with farm animals in more detail see table 6. Def: Definition

#Age of subjects when assessment of health outcome was done. <sup>a</sup>Mean age; AR: Allergic rhinitis/rhinoconjunctivitis; HF: Hay fever; AE: Atopic eczema; AA: Atopic asthma; e: Eczema

DD: Doctor diagnosed; SR: Self reported; SRS: Self reported symptoms; 12: Past 12 months; LF: Lung function; i: Incidence

↓: Statistically significant inverse association ↑: Statistically significant positive association ↓ or ↑: Direction of statistically non-significant association, regardless of effect size

n.s. Statistically not significant association, direction of the effect not reported. (P) Prospective longitudinal study



**Table 4.** Summary of studies investigating associations between living in a farm environment\* during childhood and allergic diseases (ever during lifetime if otherwise not mentioned) in adulthood.

Ref.	Year	N	Age#	Asthma		AR/HF		AE	
				Def.	Results	Def.	Results	Def.	Results
Health assessment in adulthood									
Kipelaïnen et al.	2000	10667	18-24	DD	↓	AR;DD	↓↓	DD	↓
Pekkanen et al. (P)	2001	5192	31	DD	↓↓	---	---	---	---
Leynaert et al.	2001	6251	20-44	SR12	↓	AR; SRS	↓	---	---
Kauffman et al.	2002	397	43 <sup>a</sup>	SRS	↓↓	---	---	---	---
Portengen et al.	2002	1386/1401	19 <sup>a</sup>	SR	↓	HF; SRS	↓↓	---	---
Kipelaïnen et al.	2002	296	19-27	SRS12+LF	↓↓	---	---	---	---
Koskela et al.	2003	466	39-41 <sup>a</sup>	---	---	AR; SRS	↓	---	---
Braback et al.	2004	1309652	17-20	DD	↓	AR;DD	↓↓	DD; e	↓
Leynaert et al.	2006	600	45 <sup>a</sup>	SR	↑	AR;SR	↓↓	---	---
Douwes et al.	2007	5616	43/49 <sup>a</sup>	DD	↓↓	HF;SR	↓↓	SR; e	↓
Schulze et al.	2007	1595	18-44	DD	↓	AR;SR	↓↓	---	---
Smit et al.	2007	1798	44/46 <sup>a</sup>	SR12	↓	HF;SR	↓↓	---	---
Hoppin et al.	2008	25814	20-88	DD; AANA	↓↓	---	---	---	---
Matheson et al. (P)	2009	3429	44	---	---	HF; SRS	↓	---	---
Eriksson et al.	2010	18087	16-75	---	---	HF;SR	↓↓	---	---
Wennergren et al.	2010	1261	16-20	DD	↓↓	---	---	---	---
Pallasaho et al.	2011	4302	20-69	DD;i	n.s.	HF; SR;i	↓↓	---	---
Matheson et al.	2011	8486	20-44	---	---	HF; SR;i	↓	---	---
Omland et al.	2011	2371	24 <sup>a</sup>	SR;i	↓↓	---	---	---	---
Varraso et al.	2012	54018	43-68	DD	↓↓	---	---	---	---

\* Living in any farm, to be born in any farm or parental farming. For studies examining at farms with farm animals in more detail see table 6.

#Age of subjects when assessment of health outcome was done; <sup>a</sup> Mean age; Def: Definition; ↓: Incidence

AR:Allergic rhinitis/rhinoconjunctivitis; HF:Hay fever; AE:Atopic eczema; AA:Atopic asthma; NA: Non-atopic asthma; e: Eczema

DD: Doctor diagnosed; SR: Self reported; SRS: Self reported symptoms; 12: Past 12 months; LF: lung function;

↓↓ Statistically significant inverse association ↑↑ Statistically significant positive association ↓ or ↑ Direction of statistically non-significant association, regardless of effect size

n.s Statistically not significant association, direction of the effect not reported. (P) Prospective longitudinal study

### 2.2.3 Allergic rhinitis

Table 3 summarizes the studies investigating associations between a farm environment and allergic rhinitis in childhood. The majority, 12 of 20, of published studies reported statistically significant inverse associations between a farm environment and allergic rhinitis during childhood (i.e. protective effect). This is in line with the meta-analysis published by Perkin et al. which stated that the pooled estimate for allergic rhinitis in childhood was 0.64 (95% CI 0.49-0.85) (Perkin et al. 2006). There appears to be only one prospective longitudinal study into this topic. Matheson et al. (2009) conducted a prospective longitudinal study and reported that there were no statistically significant associations between paternal farming when the child was aged 7 and early onset allergic rhinitis (before age of 7) or late onset allergic rhinitis (developed between age of 7 and 44 years).

The preventive effect of childhood farm environment seems to continue into the adulthood with 9 of 13 published studies reporting statistically significant inverse associations between a farming environment during early childhood and allergic rhinitis during adulthood (i.e. a protective effect) (table 4). Braback et al. (2004) conducted a large register cohort study and found significant inverse associations between the childhood farm environment and allergic rhinitis in adulthood. Matheson et al. (2011) reported longitudinal evidence from European Community Respiratory Health Study. Exposure to a farm environment during early childhood was a protective factor for incident rhinitis in adolescence but not in adulthood and exposure information was collected retrospectively in the study.

A few studies have also observed associations between prenatal exposure to a farming environment and allergic rhinitis. A cross-sectional study by Riedler et al. (2001) reported that children whose mothers were active on the farms during pregnancy had a lower risk of suffering hay fever in comparison to children with non-active mothers. However, Ege et al. (2006) reported that prenatal exposure to stables was not statistically associated with allergic rhinoconjunctivitis with similar associations being described in a recently published cross-sectional study (Lee et al. 2012). In contrast, the cross-sectional study conducted by Douwes et al. (2008) reported that prenatal farm animal exposure did protect from hay fever. However, combination of prenatal and current exposure was most protective against hay fever in the study.

In conclusion, living in a farming environment during childhood may protect from development of allergic rhinitis and the preventive effect seems to continue into the adulthood, although there is lack of prospective longitudinal studies. The

effect of prenatal exposure to the farming environment during pregnancy has remained unclear.

#### 2.2.4 Atopic eczema

Although living in a farm environment during childhood seems to protect from atopy, asthma and allergic rhinitis, as reviewed above, the situation for atopic eczema is not consistent. There are only two cross-sectional studies reporting statistically significant inverse associations between living in a farm environment and atopic eczema in childhood (Douwes et al. 2008, Dimich-Ward et al. 2006). Most of the studies have found weak, statistically non-significant, associations without any consistent direction (table 3 and table 4).

It seems that there are only two prospective longitudinal studies on the matter published to date. In the prospective longitudinal study conducted by Benn et al. (2004), there was a weak non-significant inverse association between living in a farming environment during infancy and the incidence of atopic eczema at the age 1.5 years. A similar association was reported in a prospective longitudinal study conducted during the first years the child's life (Roduit et al. 2011). In the large register cohort study, a statistically significant inverse association was detected between farming environment during childhood and the risk of eczema during adulthood. However, this association was only seen in the cohort 1972-1981 but not in earlier cohorts (Braback et al. 2004).

A few studies have reported associations between the prenatal exposure to the farming environment and the development of atopic eczema. Douwes et al. (2008) reported that prenatal farm animal exposure could protect from eczema during childhood. However, combination of current and prenatal exposure was most protective against eczema (Douwes et al. 2008). Similar associations between prenatal exposure to the farm animals and atopic eczema has been described in a recently published prospective birth cohort study (Roduit et al. 2011) as well as in cross-sectional study (Lee et al. 2012).

In conclusion, there is no consistent evidence that living in a farm environment during childhood can affect the risk of atopic eczema. In line with this proposition, in the meta-analysis conducted by Perkin et al. (2006) no association was reported between a farming environment during childhood and the risk of current atopic eczema during childhood.

## 2.3 Farm animals, farm milk and other mediating factors related to farm environment in early childhood

There are several potential environmental risk factors for asthma or atopy such as smoking, passive smoking, air pollution, weight, diet, infections during early childhood and environmental microbial exposure (Eder et al. 2006). The farming environment also includes numerous lifestyle and environmental features which are different from those encountered in the urban environment. Several studies have tried to determine which features in farm environment are essential for the protective effect against atopy and allergic diseases. In Finland, Remes et al. (2003) have examined in detail differences in the environment, e.g household pets, number of older siblings, fireplace use, diet, maternal smoking during pregnancy and farm milk consumption, and lifestyle-related factors between children of farmers and non-farmers. However, differences were mostly not associated with atopy apart from contact with either farm animals or pets during infancy (Remes et al. 2003). Studies investigating associations of farm-related exposures, especially those associated with environmental microbial exposure such as farm animals and farm milk consumption, with atopy and allergic diseases will be reviewed in the following chapters.

### 2.3.1 Farm animal related exposures and atopy

Table 5 summarizes the published studies investigating the associations of childhood farm animal contact and/or farm milk consumption with atopy. In 9 of 16 studies statistically significant inverse associations were observed for farm animal contact with atopy and in 7 out of 14 studies statistically significant inverse associations were found between farm milk consumption and atopy.

Riedler et al. (2001) has reported a decreased risk of atopy in children exposed to stables and farm milk in early childhood with both exposures exerting independent protective effects. Furthermore, there was also a dose-dependent and inverse association between exposure to the stables in first years of life and the risk of atopy in childhood (Riedler et al., 2001). A similar dose-dependent and inverse association with farm animal contact during early childhood has been described by Remes et al. (2003).

von Hertzen et al. (2006) observed inverse associations between contact with farm animals and the risk of atopy. However when Russian and Finnish children were analysed separately the statistically significant association was restricted to Finnish children (von Hertzen et al. 2006). In a cross-sectional study, a decreased risk atopy was associated with contact with pigs in early childhood. However, current exposure to the pigs increased risk of atopy in exposed children (Wickens et al. 2002). Sozanska et al. (2014) reported recently that continuous contact with pigs or poultry, however not with cows, could protect from new atopy in population with subjects of

all ages. However, in that study, the effect of childhood and adulthood exposure could not be differentiated (Sozanska et al. 2014). In a prospective birth cohort study, farm animal contact in early childhood was associated with the increased incidence of sensitization to perennial allergens at the age of one year (Depner et al. 2013).

In a cross-sectional study, farm milk consumption was protective against atopy during childhood but it explained only part of the overall protective effect of the farm environment (Waser et al. 2007). A recent study claimed that there was decreased risk of atopy in subjects with farm milk consumption during their early years. This study population included subjects of all ages. The associations were most evident in children in comparison to adults and in non-farmers as compared to farmers. (Sozanska et al. 2013). In the study of Loss et al. (2011) and Perkin et al. (2006) the protective effect of farm milk consumption was independent of other farm-related factors. It has proven difficult to assess different mediating factors related to the farming environment due to mutual correlations and overlapping of exposures (Illi et al 2012). Illi et al. (2012) reported numerous different exposures in farms which displayed inverse associations with atopy. After multivariate analysis, farm milk consumption, contact with straw and farming remained protective. Farm milk consumption remained inversely associated with atopy independently from farm animal contact. The authors of that study suggested that the protective effect of farm milk consumption and farm animal contact may be mediated through different biological pathways (Illi et al. 2012). Similarly, numerous protective farm-related factors from the Polish center of the same study were reported. After multivariate analysis, contact with poultry (atopy assessed with serum IgE-antibodies) and contact with grains (atopy assessed with skin prick tests) remained and seemed to confer protection against atopy (Macneill et al.2013).

Contact with farm animals during childhood may also protect from atopy during adulthood. Radon et al. (2004a) described inverse associations of farm milk consumption and farm animal contact in childhood with atopy in adulthood with both exposures exerting independent effects. In another cross-sectional study, farm animal contact in childhood was inversely associated with atopy in adulthood. (Radon et al. 2006b).

In conclusion, there is evidence that farm animal contact and farm milk consumption in early childhood may protect from the development of atopy during childhood. However, the effect of contact with farm animals and farm milk consumption in early childhood may be mediated via different biological pathways since these factors seem to exert independent effects (Illi et al.2012). The effect of farm animal contact in childhood and atopy in adulthood has been investigated in only a few studies.

**Table 5.** Summary of studies investigating associations between farm animal contact and/or farm milk consumption in early childhood and atopy as assessed with skin prick tests (SPT) or serum IgE-antibodies (IgE) for specific allergens in different age groups.

Ref	Year	N	Age#	Method	Farm animal contact*	Farm milk consumption*
<i>Health assessment in childhood or adolescence</i>						
Riedler et al.	2001	901	6-13	IgE	↓ ↓	↓ ↓
Barnes et al.	2001	929	11-19	SPT	↓ ↓	↓ ↓
Wickens et al.	2002	275	7-10	SPT	↓ ↓ 1y / ↑↑ current	↓
Renes et al.	2003	710	6-13	SPT	↓ ↓	↓
Ege et al.	2006,2007	2086	5-13	IgE	↓	↓
von hertzen	2006	813	7-16	SPT	↓ ↓	-----
Zekveld et al.	2006	797	7-18	SPT	↑	n.s.
Perkin et al.	2006	879	children	SPT	↓ ↓	↓ ↓
Waser et al.	2007	3979	5-13	IgE	-----	↓ ↓
Ma et al.	2009	1127	13-14	SPT	↑	-----
Loss et al.	2011	7606	6-12	IgE	-----	↓ ↓
Lee et al.	2012	1507	9 - 12	SPT	↑	-----
Illi et al.	2012	7682	9 <sup>a</sup>	IgE	↓	↓ ↓
Macneil et al.	2013	2586	9 <sup>a</sup>	IgE/SPT	↓/↓ ↓	↓
Cooper et al.	2013	6821	5-16	SPT	↑	↑
Depner et al. (P)	2013	793	1	IgE	↑↑↑	-----
<i>Health assessment in adulthood</i>						
Radon et al.	2004a	321	18-44	IgE	↓ ↓	↓
Radon et al.	2006b	2678	18-44	IgE	↓ ↓	-----
Sozanska et al.	2013	1664	5-adulthood	SPT	-----	↓ ↓

#Age of assessment of allergic sensitization; <sup>a</sup>Mean age; \*Sensitization to any allergen studied if otherwise not mentioned

↓ ↓ Statistically significant inverse association ↑↑ Statistically significant positive association; ↓ or ↑ Direction of statistically non-significant association, regardless of effect size

n.s Statistically not significant association, direction of the effect not reported.

### 2.3.2 Farm animal related exposures and allergic diseases

Table 6 summarizes the studies investigating the associations between farm animal contact during early childhood and allergic diseases. Only the 5 of 14 studies listed in table 6 have reported statistically significant inverse association of farm animal contact in early childhood with asthma. In contrast, a slight majority of investigations, 7 of 12 in table 6, have reported statistically significant inverse association of contact with farm animals in early childhood with allergic rhinitis. However, the risk of atopic eczema does not seem to be associated with farm animal contact during childhood, since only one of the 10 published studies has detected statistically significant inverse association (table 6).

In their cross-sectional study, Riedler et al (2001) reported that those children who had been exposed to stables and farm milk in early childhood had a decreased risk of hay fever and asthma in childhood with both exposures exerting independent protective effects. Furthermore, there was also a dose-dependent and inverse association with exposure to the stables in first years of life and the risk of hay fever in childhood (Riedler et al. 2001). Similarly, von Ehrenstein et al. (2000) observed that increasing contact with farm animals reduced the risk of experiencing atopic diseases during childhood. The protective effect of farm animal contact in early childhood may also continue to adulthood. In their cross-sectional study, Radon et al. (2004b) claimed that farm animal contact during childhood could confer protection from allergic rhinitis and atopic asthma at adult age independently from the impact of living in a farm environment during adulthood.

However, all farm animal related exposures in childhood may not be protective against allergic diseases. Sigurdson et al. (2006) reported that children attending a school near to concentrated animal feeding operations had an increased risk of suffering asthma. These authors suggested that the effect may have been due to associated environmental toxicants (Sigurdson et al. 2006). Brunner et al. (2005) found no difference in the prevalence of asthma between children living in crop farms compared to children living in livestock farms. Recently in a cross-sectional study farm animal contact during childhood was associated with increased risk of allergic rhinitis independently from paternal farming (Cooper et al. 2013). In contrast, in a study conducted among adult farmers, living in a farm with livestock during childhood tended to be inversely associated with having allergic asthma during childhood. However, living in a farm with vineyards, fruit or vegetables was positively associated with childhood allergic asthma. The information about farm environment during childhood was collected retrospectively at adult age (Baldi et al. 2014).

Table 7 summarizes the studies which have investigated the associations between farm milk consumption during childhood and allergic diseases with 5 of 8 studies describing statistically significant inverse associations between farm milk consumption in early childhood and asthma. Similarly, 5 of 9 studies detected statistically significant inverse associations with allergic rhinitis (table 7). In their cross-sectional studies, Waser et al. (2007) and Loss et al. (2011) described an inverse association between farm milk consumption and allergic diseases in childhood independently from other farm-related factors (Waser et al. 2007; Loss et al. 2011).

Illi et al. (2012) conducted a cross-sectional study and examined numerous exposures in farms with inverse associations with asthma and allergic rhinitis. However in the multivariate models, farm milk consumption, contact with cows and farming remained protective. The authors of the study suggested that protective effect of farm milk consumption and farm animal contact may be mediated by different biological pathways as the effects were independent and the exposure routes may be different (Illi et al. 2012).

In conclusion, farm animal contact and farm milk consumption may confer protection from development allergic rhinitis in childhood. In addition, there has been a similar suggestion of a protective effect against the development of asthma childhood. The effects of farm animal contact and farm milk consumption may be mediated via different biological pathways since they seem to have independent effects (Illi et al. 2012). The effects of farm animal contact or farm milk consumption in childhood and asthma and allergic diseases in adulthood have been reported in only a few studies.



**Table 6.** Summary of studies investigating associations between farm animal contact in early childhood and allergic diseases (ever during lifetime if otherwise not mentioned) in different age groups.

Ref.	Year	N	Age#	Asthma		AR/HF		AE	
				Def.	Results	Def.	Results	Def.	Results
Health assessment in childhood or adolescence									
Riedler et al.	2001	2618	6-13	DD	↓↓	HF; DD	↓↓	---	---
Wickens et al.	2002	293	7-10	SR	↑	HF; SR	↓	SR	↓
Remes et al.	2002	7981	13-14	DD	n.s.	HF; SR	↓	SR	↓
Merchant et al.	2005	644	1-17	DD	↑	---	---	---	---
Salam et al.	2004	691	5	DD	↑	---	---	---	---
Ege et al.	2006;2007	8263	5-13	DD	↓↓/↑	HF; DD	↓↓	---	---
Dimich-ward et al.	2006	1158	8-20	DD	↓	AR; SRS;12	↓↓	SRS;12	↓↓
Karadaq et al.	2007	14893	5-13	---	---	---	---	DD	↑
Douwes et al.	2008	1899	5-17	SR	↓↓	HF; SR	↓↓	SR; e	↓
Ma et al.	2009	3525	13-14	DD	↓	AR; SRS;12	↑	SRS;12	↑
Illi et al.	2012	8419	9 <sup>a</sup>	SR	↓↓	HF; SR	↓↓	DD	↓
Lee et al.	2012	1749	9 - 12	DD	↓	AR; DD	↓	DD	↑
Cooper et al.	2013	6821	5-16	---	---	AR; SRS	↑↑	SRS	↑
Barry et al.	2014	2259	6-18	DD	↓	---	---	---	---
Health assessment in adulthood									
Radon et al.	2004b	3112	18-44	SRS; NA/AA	↑↑/↓↓	HF; SR	↓↓	SRS	↔
Smit et al.	2007	1798	44/46 <sup>a</sup>	SR12	↓	HF; SR	↓↓	---	---

#Age of subjects when assessment of health outcome was done; Def: Definition; n:Mean age

AR:Allergic rhinitis/rhinoconjunctivitis; HF:Hay fever; AE:Atopic eczema; AA:atopic asthma; NA: non-atopic asthma; e: Eczema

DD: Doctor diagnosed; SR: Self reported; SRS: Self reported symptoms; 12: Past 12 months; LF: Lung function;

↓ Statistically significant inverse association ↑ Statistically significant positive association ↓ or ↑ direction of statistically non-significant association, regardless of effect size ↔ no association

n.s statistically not significant association, direction of the effect not reported.

**Table 7.** Summary of studies investigating associations between farm milk consumption in early childhood and allergic diseases (ever during lifetime if otherwise not mentioned) in different age groups.

Ref.	Year	N	Age#	Asthma		ARI/HE		AE	
				Def.	Results	Def.	Results	Def.	Results
<i>Health assessment in childhood or adolescence</i>									
Riedler et al.	2001	2618	6-13	DD	↓ ↓	HF; DD	↓ ↓	-----	-----
Wickens et al.	2002	293	7-10	SR	↓	HF; SR	↑	SR	↓ ↓
Ege et al.	2006, 2007	8263	5-13	DD	↓ ↓	HF; DD	↓ ↓	-----	-----
Karadağ et al.; Vaser et al.	2007, 2008	14893	5-13	DD	↓ ↓	DD	↓ ↓	DD	↓
Douwes et al.	2008	1899	5-17	SR	↓	HF; SR	↓	SR; e	↓
Perkin et al.	2006	4767	childhood	SRS12	↓	AR; SRS; 12	↓ ↓	SRS; 12	↓ ↓
Loss et al.; Illi et al.	2011; 2012	8339/8419	6-12	SR	↓ ↓	HF; SR	↓ ↓	DD	↓ ↓
Cooper et al.	2013	6821	5-16	----	----	AR; SRS	↑	SRS	↑
<i>Health assessment in adulthood</i>									
Sozanska et al.	2013	1664	5-adulthood	DD	↓ ↓	HF; DD	↓	-----	-----

#Age of subjects when assessment of health outcome was done; Def: Definition; #Mean age  
AR: Allergic rhinitis/rhinoconjunctivitis; HF: Hay fever; AE: Atopic eczema; e: Eczema  
DD: Doctor diagnosed; SR: Self reported; SRS: Self reported symptoms; 12: Past 12 months; LF: Lung function;  
↓ ↓ Statistically significant inverse association ↑ ↑ Statistically significant positive association ↓ or ↑ Direction of statistically non-significant association, regardless of effect size  
n.s Statistically not significant association, direction of the effect not reported.

### 2.3.3 Contact with pets, atopy and allergic diseases

Contact with pets, such as cats or dogs, may also affect the risk of atopy. In a review published in 2005 the authors concluded that the effect of cat ownership on atopy was inconsistent, but the effect of dog ownership was more consistent and may be protective (Simpson et al. 2005). Similarly a recent review concluded that exposure to dogs during infancy may confer protection against sensitization to outdoor aeroallergens (Chen et al. 2010). A recent meta-analysis, which included 11 prospective birth cohorts, reported that dog ownership in early childhood could be protective against sensitization to aeroallergens (Carlsen et al. 2012).

The studies focusing on the protective effect of the farm environment, which have already been reviewed previously in this thesis (see 2.2.1 and 2.3.1), have mainly observed weak associations between dog or cat contact in early childhood and atopy (e.g. Barnes et al. 2001; Wickens et al 2002; Radon et al 2004; Cooper et al. 2013; Lee et al. 2012). However, in a few studies, the inverse associations have been statistically significant (Leynaert et al. 2001, Remes et al 2003; Von Hertzen et al. 2006, Illi et al. 2012).

The effect of pet ownership and development of allergic diseases have also been comprehensively reviewed recently. The authors of the review concluded that cat or dog ownership in early childhood had no effect on the development of asthma during childhood (Chen et al. 2010). There are also a few meta-analyses on this topic. In one recent meta-analysis with 11 European prospective birth cohorts, no statistically significant associations were detected between dog or cat ownership and the development of asthma or allergic rhinitis in childhood. There was a weak trend that dog ownership could protect from the development of asthma. However this did not reach statistical significance (Carlsen et al. 2012). In another meta-analysis, the relative risk of asthma associated with dog ownership was 1.14 in all studies and for cat ownership 0.72 in cohort studies (Takkouche et al. 2008). However, the study has been criticized in terms of health outcome and exposure definitions (Chen et al. 2009). In another meta-analyses published in 2001 there was an increased risk of asthma associated with pet ownership but the effect was relatively small (OR 1.11) (Abelberg et al. 2001). The conclusion from these published reviews and meta-analyses is that no clear and consistent trends can be detected on the effect of pet ownership and development of asthma and allergic diseases.

Studies focusing on the protective effect of the farming environment, which have already been reviewed previously in this thesis (see 2.2.2, 2.2.3, 2.2.4, and 2.3.2), have mainly observed weak associations between dog or cat contact in early childhood and allergic diseases (e.g. Merchant et al. 2004, Sigurdson et al.

2006, Salam et al. 2004, Illi et al. 2012, Leynaert et al. 2001). However, in a few studies, the inverse associations have been statistically significant (Wickens et al 2002, Lee et al. 2012, Matheson et al. 2011, Benn et al. 2004, Karadağ et al 2004). In their cross-sectional study, Cooper et al (2013) reported that the presence of pets in a household were positively associated with allergic rhinitis and atopic eczema during childhood. A recent prospective birth study suggested that prenatal contact to cats could confer protection from development of atopic eczema in early childhood (Roduit et al. 2011).

In conclusion, contact with pets during childhood, especially contact with dogs, may offer protection against development of atopy. However, contact with pets does not seem to be consistently associated with the risk of allergic diseases.

#### **2.3.4 Role of microbial exposure**

The protective effect of farming environment against atopy and asthma has been hypothesized to be related to the differences in exposure to environmental microbes between children living in farming environment and their peers living in more urban environments (von Mutius et al. 2000; Braun-Fahrlander et al. 2002; Schram et al. 2005; Ege et al. 2011). Often in epidemiologic studies, bacterial components such as endotoxin (gram-negative bacteria), muramic acid (Gram-positive bacteria) or fungal components, are used as markers of microbial exposure instead of viable bacterial or fungal species. However, there is an enormous variety of microbes and microbial agents present in house dust and these highly mutually correlate, thus it is hard to distinguish their protective effects (Gehring et al 2007).

Recently, studies investigating the association between house dust endotoxin levels and atopy have been reviewed comprehensively. The review concludes that endotoxin exposure in homes may protect from atopy, however the evidence is not consistent. The protective effect of endotoxin is much clearer when CD14 (pattern recognition receptor of innate immunity) genotype is taken into account, indicating that there may be some kind of gene-environment interaction. However, the authors reminded that other microbial or environmental factors may also be active and endotoxin may simply be a “marker for hygiene” (Simpson et al. 2010). Mendy et al. (2011) published a meta-analysis investigating associations of endotoxin exposure, measured from house dust, with asthma and wheezing in different ages during childhood. A meta-analysis revealed that current endotoxin exposure protected from asthma when children were of school-age (OR 0.82), but early childhood exposure increased the risk of wheezing during infancy (OR 1.48). (Mendy et al. 2011).

Recently, various studies have investigated the longitudinal effects of microbial exposure in homes on atopy and allergic diseases. In a prospective birth cohort study, Douwes et al. (2006) reported that exposure to microbial agents measured from house dust, such as endotoxin and fungal components during infancy were associated with lower risk of asthma at age of four. Furthermore, the associations between microbial agents and atopy were somewhat weaker (Douwes et al. 2006). In another prospective birth cohort study endotoxin exposure in inner-city homes was a protective factor against atopic eczema but a risk factor for wheezing in early childhood (Perzanowski et al. 2006). In a longitudinal study, Celedon et al. (2007) reported that endotoxin exposure in homes during infancy was associated with a decreased risk of atopy and an increased risk of wheezing in school age among children with parental atopy. In their study Bertelsen et al. (2010) stated that early childhood exposure to endotoxin or fungal components was not associated with asthma or atopy at the age of 10. Similar statistically non-significant associations with atopy have been also reported in other longitudinal studies (Chen et al. 2008, Gillespie et al. 2006). In the birth cohort study endotoxin exposure, measured from home dust, was associated with a decreased risk of atopy and asthma at school-age. In the same study, exposure to muramic acid was inversely associated with asthma but not with atopy. Furthermore, the exposure during school-age had an independent protective effect after adjustment of early childhood endotoxin exposure (Sordillo et al. 2010). Similarly, Tischer et al. (2011) reported that the levels of microbial components in house dust were inversely associated with doctor-diagnosed asthma and allergic rhinitis at age of 6. However, this association was seen in the German but not in the Dutch population of the study and the authors could not explain the difference between these populations (Tischer et al. 2011).

A few studies have tried to separate effect of microbial exposure and farming environment. In the cross-sectional study endotoxin exposure in homes was associated with a decreased risk of hay fever and atopic asthma in childhood and this was independent from living on a farm during first year of life (Braun-Fahrlander et al. 2002). Another cross-sectional study reported an inverse association between exposure to the microbial agents in homes with atopy and asthma in childhood, independently from living in a farm environment (Ege et al. 2007).

Recently, a few studies have investigated the effect of microbial diversity. Ege et al. (2011) published an article with combined data from two large cross-sectional studies. Microbial diversity in homes reduced the risk of asthma in childhood and this explained most of the protective farm effect (Ege et al. 2011).

Another cross-sectional study detected an inverse association between environmental biodiversity and atopy in childhood (Hanski et al. 2012)

In conclusion, the quantity and/or quality of microbial exposure in homes may confer protection from atopy and allergic diseases in childhood and it offers a plausible mechanism for the protective effect of farm environment. However the protective effect is not consistent and may be modified by gene-environment interactions.

## **2.4 Farming exposure in adulthood, atopy and allergic diseases**

### **2.4.1 Atopy**

Living in a farm environment during early childhood may protect from atopy, as described above (see chapter 2.2.1). The protective effect may be most evident during infancy and the effect seems to continue into adulthood. However, the association of exposure to the farming environment during adulthood with atopy is less clear. Table 8 summarizes those studies which have investigated the associations between farm environment in adulthood and atopy. In fact, only 3 out of the 10 studies listed in table 8 reported statistically significant inverse associations between farm environment in adulthood and atopy (table 8). The studies mainly involved cross-sectional designs.

One cohort study, reported that both being a farmer and living in a farm environment during childhood independently decreased the risk of atopy (Portengen et al. 2002). In a follow-up of the same cohort study, Elholm et al. (2013) reported that the farm environment during adulthood protected from new onset atopy. This association was independent from living in a farm environment as a child. In the study, the information about childhood farm environment was collected retrospectively from the subjects when they were adults (Elholm et al. 2013). Koskela et al. (2005) suggested that living in a farm environment during adulthood may protect from sensitization to common allergens even after childhood but at the same time it can be a risk factor for sensitization to farm allergens. However, the combined effect of childhood and adulthood farm environment offered the best protection against sensitization to common allergens. There was also a dose-dependent association between contact with farm animals in adulthood and sensitization to pollens (Koskela et al. 2005).

However, some studies have reported that exposure to a farm environment during adulthood could increase the risk of atopy. Chatzi et al. (2005) reported increased risk of atopy among grape farmers in Crete rural population (Chatzi et

al. 2005). One cross-sectional study claimed that there was a higher risk of asymptomatic sensitization to common allergens in those subjects whose contact with farm animals only started during adulthood (Radon et al. 2006b). Another study reported that the number of animal houses in the neighbourhood was not associated with atopy (Radon et al. 2007). In the cross-sectional study conducted by Eduard et al. (2004a) no difference was detected in the risk of atopy when crop farmers and livestock farmers were compared.

In conclusion, there is a suggestion that exposure to the farming environment during adulthood, independently from living in a farming environment in early childhood, may protect from atopy. Furthermore, continuous exposure from birth till adulthood may provide the best protection. However, the protective effect is more consistently associated with the childhood farm environment.

**Table 8.** Summary of studies investigating associations between exposure to a farm environment\* during adulthood and atopy as assessed with skin prick tests (SPT) or serum IgE-antibodies for specific allergens.

Ref.	Year	N	Age#	Def.	Atopy <sup>a</sup>
Filiapik et al.	2000	4243	25-75	IgE	↓
Kauffman et al.	2002	805	43**	SPT	↑
Portengen et al.	2002	1387/439	19-20**	SPT/IgE	↓↓
Eduard et al.	2004b	3184	21-69	IgE	↔
Chatzi et al.	2005	220	25-70	SPT/IgE	↑↑/↑
Chen et al.	2007	2081	18-79	SPT	↓↓
Koskela et al.	2005	433	39/42**	SPT	↓
Radon et al.	2006b	2678	18-44	IgE	↑
Kim et al.	2008	2118	11-71	SPT	↓
Elholm et al. (P)	2013	1166	33**	SPT/IgE	↓↓i

\* Living in any farm or occupational farming. #Age of assessment of allergic sensitization

\*\*Mean age; <sup>a</sup>Sensitization to any allergen studied if otherwise not mentioned, i: Incidence

↓↓ Statistically significant inverse association ↑↑ Statistically significant positive association

↓ or ↑ Direction of statistically non-significant association, regardless of effect size

n.s Statistically not significant association, direction of the effect not reported.

↔ No association (P) Prospective longitudinal study

## 2.4.2 Allergic diseases

Living in a farm environment during early childhood may protect from development of allergic diseases, and the effect seems to continue into adulthood as described previously (see chapters 2.2.2, 2.2.3). However, the association of exposure to the farm environment during adulthood with allergic diseases is less clear. The few reports detecting statistically significant inverse associations of

exposure to the farm environment during adulthood with allergic diseases are presented in table 9.

One cross-sectional study reported that the protective effect of living in a farm environment on asthma during adulthood was most evident in those subjects with combined childhood and adult exposure to the farm environment. However, this kind of association was not seen with allergic rhinitis or eczema (Douwes et al. 2007). Eduard et al. (2004b) reported that practicing farming in adulthood was associated with a lower prevalence of asthma. However, in that study there was no information available on the childhood farm environment (Eduard et al. 2004b). Another cross-sectional study found that living in a farm environment during adulthood was associated with the reduced risk of nasal allergies and atopic eczema but not asthma symptoms and that this effect was independent from childhood farm animal contact (Radon et al. 2004b). Koskela et al. (2003) suggested that exposure to a farm environment in adulthood may confer protection from pollen-induced upper airways symptoms independently from farm environment during early childhood. However, the effect was statistically significant only in subjects with combined childhood and adult farm exposure. Furthermore, contact with cows at adult age was dose-dependently and inversely associated with allergen induced upper-airway symptoms but increased the risk of farm work-related upper airway symptoms (Koskela et al. 2003)

Studies with positive association between exposure to the farm environment in adulthood and allergic diseases are also published. In their large population based study, Kogevinas et al. (1999) reported that farming as an occupation was associated with an increased risk of asthma. Similarly, Jenkins et al. (2004) reported that farming was a risk factor for asthma. However, neither of these studies provided any information about whether the subjects had lived in a farm environment during childhood (Jenkins et al. 2004). In a recent prospective longitudinal study, with adult farming school students, there was an increased risk of new asthma in subjects with current exposure to dairy or swine confinements. Childhood farm environment was associated with a reduced risk of asthma. In that study, the information about living in a farm environment during childhood had been collected retrospectively at the baseline (Omland et al. 2011). Chatzi et al. reported in 2005 that current grape farming was positively associated with allergic rhinitis and that there was also similar suggestion towards asthma. Furthermore, Radon et al. (2007) reported that the number of animal houses in the neighbourhood was not statistically significantly associated with allergic rhinitis or asthma in adulthood.



A few studies have investigated associations between farm-related exposures and allergic diseases within farmer populations. In a cross-sectional study combined exposure to the farm environment in childhood and livestock farming at an adult age conferred the best protection against hay fever. However, no such similar association was seen for asthma (Smit et al. 2007). Eduard et al. (2004a) reported that swine and cattle farmers had a higher risk of asthma than crop farmers. When the study population was stratified according to the presence of atopy, farm animals tended to decrease the risk of asthma among atopic farmers and increase the risk of asthma among their non-atopic counterparts (Eduard et al. 2004a). Another cross-sectional study found that allergic diseases were more common among farmers working with farm animals (Kimbell-Dunn et al. 1999). Radon et al. reported that pig farmers had the highest risk for developing work based respiratory symptoms as compared other types of farming. In the same study, the authors compared asthma prevalence in the farmer population with the prevalence in general population. In contrast, there was a lower prevalence of asthma and nasal allergies in the farming population (Radon et al. 2001).

In conclusion, there is no consistent evidence that exposure to the farming environment during adulthood, independently from growing up on a farm can protect from allergic diseases. Farming as an occupation, especially working with farm animals, may even increase the risk of asthma.

**Table 9.** Summary of studies investigating associations between exposure to a farm environment\* during adulthood and allergic diseases (ever during lifetime if otherwise not mentioned).

Ref.	Year	N	Age#	Asthma		AR/HE		AE	
				Def.	Results	Def.	Results	Def.	Results
Kogevinas et al.	1999	15637	22-44	SRS12	↑†				
Fillapik et al.	2001	6481	25-75	SRS12	↓	HF;SR	↓		
Kaufman et al.	2002	397	43 <sup>a</sup>	SRS	↓				
Portengen et al.	2002	1386/1401	19-20 <sup>a</sup>	SR	↓	HF; SRS	↑		
Koskela et al.	2003	466	39-41	DD	↓	AR; SRS	↓		
Eduard et al.	2004b	3184	21-69	DD	↓				
Radon et al.	2004b	3112	18-44	SRS; NA/AA	↓/↓	HF; SR	↓	SR	↓
Jenkins et al.	2004	11272	over 45	DD	↑†				
Chatzi et al.	2005	220	25-70	SRS12+LF	↑	AR; SRS12	↑†		
Douwes et al.	2007	5616	43/49 <sup>a</sup>	DD	↓	HF; SR	↓	SR <sup>e</sup>	↓
Chen et al.	2007	2081	18-79	DD	↓				
Sigurdson et al.	2008	1796	adult	DD	↓	AR; DD	↓		
Hoppin et al.	2008	25814	20-88	DD;AA	↑				
Onland et al. (P)	2011	2371	24 <sup>a</sup>	SR	↑††				

\*Living in any farm or farming occupation. #Age of subjects when assessment of health outcome was done. <sup>a</sup> Mean age. Def. Definition  
AR:Allergic rhinitis/rhinoconjunctivitis; HF:Hay fever; AE:Atopic eczema; AA:Atopic asthma; NA: Non-atopic asthma; e: Eczema  
DD: Doctor diagnosed; SR: Self reported; SRS: Self reported symptoms; 12: Past 12 months; LF: Lung function; †: Incidence  
↓ or ↑ Statistically significant inverse association †† Statistically significant positive association  
↓ or ↑ Direction of statistically non-significant association, regardless of effect size; n.s Statistically not significant association, direction of the effect not reported.  
(P) Prospective longitudinal study

## 2.5 Farming and lung function

Respiratory health problems such as asthma, allergic rhinitis, organic dust syndrome and extrinsic allergic alveolitis are well known occupational health problems found in farmers (Linaker et al. 2002). Continual environmental exposures in stables and farms, such as dusts, chemicals, gases, fumes, and infectious agents (Linaker et al. 2002) can induce irritation and evoke inflammation in the respiratory system (Bardana et al. 2008; Radon et al. 2006a, Linaker et al. 2002) and lead to lung function impairment (as reviewed in the following chapters). Therefore these environmental exposures are potentially risk factors for respiratory diseases (Linaker et al. 2002; Bardana et al. 2008; Radon et al. 2006a). On the other hand, living in a farm environment during infancy is associated with decreased risk of atopy and allergic diseases (see chapters 2.2.1, 2.2.2, 2.2.3, 2.3.1 and 2.3.2). Therefore, living in farm environment during infancy may reduce the risk of allergic diseases in adulthood, whereas occupational exposure during adulthood may be a risk factor for respiratory diseases. However, only a few studies have reported associations between childhood farm environment and lung function.

In epidemiologic studies, lung function is often measured with spirometry. FEV1 (Forced expiratory volume in one second) expresses the volume of expired air during the first second of forced expiration. FVC (Forced vital capacity) expresses the total volume of air expired (Miller et al. 2005). FEV% (Ratio of FEV1/FVC) is used to assess the possibly airflow obstruction (Pellegrino et al. 2005).

### 2.5.1 Living on a farm in early childhood

There are only few studies published which have investigated the associations between farm environment during early childhood and lung function. In their cohort study, Merchant et al. (2005) reported that children born in a farm had higher FVC than children not born in that kind of home. However, the farm environment was not associated with FEV1 or FEV% (Merchant et al. 2005). In a recent cross-sectional study, farm environment was not associated with lung function in childhood (Fuchs et al. 2012). Ernst et al. (2000) stated that there was no difference in the FEV1/FVC ratio among adolescents raised on a farm as compared to other adolescents. Furthermore, in a cross-sectional study, there were no significant associations between the farm environment in early childhood and lung function during adulthood, as assessed by FEV1/FVC ratio (Schulze et al. 2007).

In conclusion, there is no strong evidence that living in a farm environment during early childhood can affect lung function. However, only a few studies have investigated this topic and apparently no longitudinal studies have been published.

### 2.5.2 Farming exposure in adulthood

Occupational associations between farming and lung function have been more widely reported. In the 1980s, Heller et al. (1986) reported that male farmers had higher FVC values than control industrial workers. In the same study, the levels of FEV1 and forced mid expiratory flow were significantly lower among farmers when FVC was taken into account to the model (Heller et al. 1986). Since then several studies have been published investigating the associations between farming and lung function.

Farming as an occupation, especially if it involves working with farm animals, has been reported to be associated with reduced lung function in cross-sectional studies, especially when assessed with FEV1, FVC or FEV1/FVC (Gainet et al. 2007; Lamprecht et al. 2007; Chatzi et al. 2005; Zhou et al. 1991; Dalphin et al. 1989; Cormier et al. 1991), as compared to non-farming subjects. It has been reported that environmental exposure to a high number of animal houses was associated with decreased FEV1 (Radon et al. 2007). However, Omland et al. (1999) reported that there was no association between occupational farming and lung function.

Longitudinal studies have detected a decline in lung function associated with farming, especially if it involves working with farm animals. An accelerated mean annual decline in farmers lung function (FEV1, FVC or FEV1/FVC) compared to non-farming controls has been reported in several studies (Senthilselvan et al. 1997; Dalphin et al 1998; Chaudemanche et al. 2003; Gainet et al. 2007). A recent longitudinal study conducted by Thaon et al. (2011) detected a greater annual decline in FEV1/FVC among dairy farmers as compared to control subjects. Chenard et al. (2007) conducted a longitudinal study and reported that those pig farmers who had quit working had significantly lower predicted values of FEV1/FVC as compared to farmers or controls, possibly due to so-called healthy worker effect.

A few studies have reported differences in lung function between different types of farmers. In a longitudinal study, farmers working with farm animals did not exhibit any acceleration in lung function decline as compared to farmers not working with farm animals (Schwartz et al.1995). One cross-sectional study reported that dairy farmers had lowered FEV1 but not values of FVC as compared to crop farmers (Eduard et al. 2009). Iversen et al. (1990) investigated the

differences in lung function between pig and dairy farmers. There was some indication that pig farming was associated with lower predicted value of FEV1 than dairy farming (Iverse et al. 1990) and also with an accelerated annual decline in FEV1 (Iverse et al. 2000). Zejda et al. (1993) reported that pig farmers had lower predicted values of FEV1, FVC and FEV1/FVC than grain farmers. However, there was no difference between grain farmers and the non-farming controls (Zejda et al. 1993).

In conclusion, farming as an occupation seems to be a risk factor for suffering an impairment of lung function. The risk seems to be associated more strongly to working with farm animals i.e. dairy and pig farming and lung function seems to decline as the exposure continues.

## 2.6 Farming and stimulated peripheral blood cytokine production

Growing up in a farm environment and especially farm-related factors associated with environmental microbial exposure such as farm animals in early childhood may protect from atopy and allergic diseases (see 2.2.1, 2.2.2, 2.2.3, 2.3.1 and 2.3.2). The preventive effect is hypothesized to be related to development of the T-cell mediated immunity, particularly T helper cell subsets  $T_H1/T_H2$  and regulatory T-cells. The complex findings behind the hypothesized immunological mechanism have been comprehensively reviewed recently. Briefly, a model has been proposed where exposure to a farm environment, especially contact with farm animals, during early childhood or during the prenatal period leads to regulatory T-cell activation, interferon-gamma (IFN- $\gamma$ ) production and  $T_H1$ -cell activation and furthermore to expression of innate immune pattern recognition receptors, such as toll-like receptors. These changes in immune system development lead to a suppression of “ $T_H2$ -cell-associated cytokine production and  $T_H2$ -cell dependent IgE synthesis” and furthermore suppression of allergic inflammation (Von Mutius et al. 2010).

Differences in cytokine production characterize  $T_H1$ -responses (IFN- $\gamma$ , interleukin-2 [IL-2], Tumor necrosis factor-beta [TNF- $\beta$ ], and reduction of  $T_H2$  cytokine production) and  $T_H2$ -responses (IL-4, -5, -9, -13 and reduction of  $T_H1$  cytokine production). Production of IL-10 is one of the characteristic for regulatory T-cell responses (Romagnani 2004). Therefore cytokine responses have been used as markers for allergic- or non-allergic-type immunological responses in epidemiologic studies. Furthermore, in immunological and epidemiologic studies it is possible to use in vitro stimulation in order to measure capacity to produce different cytokines in response to different types of stimulants, such as lipopolysaccharide (LPS, activation of innate immune responses) and Gram-

positive bacterial superantigen (SEB, to activate T-cells and innate immunity) (Calkins et al. 2002; Mandron et al. 2006).

### 2.6.1 Living on a farm in early childhood and cytokine production

In the past decade, a few attempts have been made to investigate the associations between exposure to a farm environment and farm-related microbial factors in early childhood and immune system development, particularly stimulated peripheral blood cytokine responses. In 2002, Braun-Fahrlander et al. reported that endotoxin exposure in house dust was inversely related to stimulated cytokine production. The authors of that study suggested that this have been attributable to down-regulation of the immune responses in the exposed children, possibly because of long-term exposure (Braun-Fahrlander et al. 2002). Roponen et al. (2005) reported that endotoxin levels in house dust, farming and cat/dog ownership were associated with stimulated peripheral blood IFN- $\gamma$  production indicating T<sub>h</sub>1-type non-allergic responses during early infancy.

Similarly, farm milk consumption has been associated with increased IFN- $\gamma$  production in childhood (Perkin et al. 2006). Lappalainen et al. described associations between house dust microbial markers and stimulated cytokine production. There was an association between exposure to gram-negative bacteria in house dust and a down-regulation of the levels of pro-inflammatory cytokines in both mothers and infants (TNF- $\alpha$  and IL-6) and the suggestion of an opposite effect with gram-positive bacteria. However, the levels of microbial markers in house dust were not associated with IFN- $\gamma$  production except bed dust endotoxin levels (Lappalainen et al. 2008). Another cross-sectional study found that high indoor microbial exposure may reduce SEB-stimulated T<sub>h</sub>1-type cytokine secretion at age of one. The authors postulated that this effect could be due to the continuous exposure to house dust endotoxin, which could lead to an adaptation of the immune system and down-regulation of cytokine production (Lappalainen et al. 2012). This theory was proposed by Braun-Fahrlander et al. in 2002.

Pfefferle et al. (2010) reported that maternal farming was associated with a significantly higher production of INF- $\gamma$  and tumor necrosis factor-alpha (TNF- $\alpha$ ) in stimulated cord blood mononuclear cells. However, there were no associations with the levels of IL-5, IL-10 or IL-12. Furthermore the mother's activity on the farm and diet during pregnancy modulated cytokine production by cord blood cells. These findings also indicate that the farming environment may be associated with T<sub>h</sub>1-type immune responses at birth (Pfefferle et al. 2010). In a recent cross-sectional study, maternal exposure to a farm environment during pregnancy was associated with increased numbers of regulatory T-cells and lower T<sub>h</sub>2 –cytokine production in cord blood (Schaub et al 2009).

In conclusion, farm-related environmental exposures during early childhood may affect the  $T_H2/T_H1$ -cell cytokine production, regulatory T-cell responses and/or down-regulation of the immune responses in childhood.

### 2.6.2 Farming exposure in adulthood and cytokine production

There is a suggestion that exposure to a farm environment during adulthood may also protect from atopy (see 2.4.1). However, the capacity of farm-related exposures in adulthood to modulate immune responses is less clear.

There appears to be only one previous population based study investigating the association between environmental microbial exposure during adulthood and stimulated peripheral blood cytokine production. Lappalainen et al. (2008) reported that the levels of gram-positive and negative bacteria, measured from house dust, were associated with stimulated peripheral blood cytokine production in the mothers that they studied. The levels of Gram-negative bacteria were associated with a down-regulation of pro-inflammatory cytokines (TNF- $\alpha$  and IL-6) whereas gram-positive bacteria had an opposite effect (Lappalainen et al. 2008).

However, non-stimulated pro-inflammatory cytokine responses have been studied more in occupational setting. Cormier et al. (2004) reported that subjects working in swine barns had higher levels of serum IL-6 (a pro-inflammatory cytokine) than controls. A Few other studies have been published describing associations between exposure to farm animals and increased production of serum pro-inflammatory cytokines (Israel-Assayag et al. 2012; Dosman et al. 2006; Bonlokke et al. 2006; Wang et al. 1998). In a study conducted in adult subjects, pig-farmers and smokers had increased IL-13 and IL-4 producing  $T_H$ -cell levels after stimulation ( $T_H2$ -responses) as compared to their non-farming controls (Shalander et al. 2010). Recently Tabibi et al. (2012) reported that occupational exposure to farm animals was associated with higher serum levels of IL-8, IL-10 and TNF- $\alpha$  than those in non-exposed controls. These studies indicate that farming environment may alter peripheral blood pro-inflammatory cytokine production during adulthood. Furthermore the inflammatory effect is probably a result from exposure to high levels of endotoxin and other organic dusts in the occupational setting (Poole et al. 2012).

In conclusion, during adulthood, the effects of the farming environment and associated microbial exposure to modulate immune system responses are less clear than those observed during childhood and the main focus has been on examining the associations between occupational exposure on farms and pro-inflammatory cytokines. However, there is a paucity of population based studies investigating

putative association between the farming environment and stimulated T<sub>h</sub>1 and T<sub>h</sub>2 cytokine responses in adulthood.

## 2.7 Conclusions from the reviewed literature

Studies, with mainly cross-sectional designs published in the past decade, have shown that living on a farm during early childhood can protect from atopy in childhood. The protective effect may even occur at the time of fetal development and even continue into adulthood. Living on a farming environment during early childhood may also protect from the clinical manifestations of allergic rhinitis and asthma. There is no consistent evidence with respect to atopic eczema, which might result from the observation that atopic eczema is not so strongly associated with atopy (Williams et al. 2006)

However, there is a lack of prospective cohort studies. Prospective longitudinal studies are needed in order to better control for recall bias and to take confounding factors into account as well as obtaining more detailed information on the persistence of symptoms/diseases which should be assessed with follow-up examinations involving objective measurements at multiple ages (Hancox et al. 2012).

Only little more than half of asthma is attributable to atopy (Pearce et al. 1999) and presence of atopy cannot be used to divide asthmatics into the allergic and non-allergic phenotypes (GINA, 2012). Nonetheless, the definition of atopic and non-atopic asthma, which is typically based on presence or absence of atopy, is currently widely used when designing and analysing studies on the determinants and characteristics of asthma.

The independent effect of farm environment during adulthood is less clear. There is a suggestion that the exposure to the farming environment during adulthood, independently from living in a farming environment in early childhood, may protect from atopy. However, continuous exposure from birth through adulthood may offer the best protection. With respect to asthma or allergic diseases there is no convincing evidence for any kind of protection. Farming as an occupation, especially working with farm animals, may even increase the risk of asthma.

The protective effect of farm environment may be due to contact with farm animals and farm milk consumption in the early years. In the studies reviewed in the previous chapters, contact with farm animals during early childhood and farm milk consumption has exerted protective effects against the development of atopy and allergic rhinitis. In addition, there has been a similar suggestion of a protective



effect against the development of asthma. The effects of farm animal contact and farm milk consumption may be mediated via different biological pathways since they seem to exert independent effects (Illi et al. 2012). The presence of household pets, such as dogs and cats, may also confer protection against atopy, but there is no consistent evidence that they are protective against asthma or allergic diseases.

The protective effect of farming environment, especially contact with farm animals, against atopy, asthma and allergic diseases is hypothesized to be related to differences in the quantity and quality of the microbial exposure. Exposure to microbes in the environment may be protective against the development of asthma and atopy, and this represents a plausible mechanism to explain why the farming environment may be beneficial. However, the protective effect is not consistent and may be modified by gene-environment interactions.

There is no strong evidence that living in a farm during early childhood can affect lung function and there are only few studies which have investigated this topic but there do not appear to be any longitudinal studies. However, there is evidence that farming as an occupation seems to be a risk factor for an impairment of lung function. The risk seems to be associated more strongly with working with farm animals i.e. dairy and pig farming and lung function seems to decline as the exposure continues. Since occupational exposure to farm animals may impair lung function but at the same time childhood exposure may be protective against allergic diseases, there is clear need for prospective longitudinal studies investigating whether living in a farm environment during childhood can effect on lung function during adulthood.

The protective effect of the farming environment is hypothesized to be related to the development of the immune system. There is evidence from the epidemiologic studies reviewed in the previous chapters, that farm-related environmental exposures during early childhood may affect the Th2/Th1-cytokine production, regulatory T-cell responses and/or down-regulate the immune responses. However, during adulthood, the effects are less clear and the main focus has placed on examining associations between pro-inflammatory cytokines and occupational exposure on farms. There is a lack of population based studies investigating the associations between the farm environment and stimulated Th1- and Th2-type cytokines responses. Thus, these complex immunological mechanisms clearly demand further investigations.



# 3 Aims of the study

This doctoral thesis examines the effect of the farm environment on atopy, allergic diseases, respiratory health and immune system responses. This thesis has four specific aims:

1. To investigate the prospective longitudinal associations of farm-related factors, especially farm animal contact, in infancy with reported allergic diseases and atopy during adulthood (I).
2. To study the prospective longitudinal associations of farming environment in infancy with lung function during adulthood (II).
3. To investigate the effect of farm-related factors on stimulated cytokine responses among adult women living on farms (III).
4. To explore whether analysing atopic or non-atopic asthma, as is typically done in epidemiologic studies, can lead to misleading associations (IV).



# 4 Methods

## 4.1 Study designs and study populations

### 4.1.1 Northern Finland Birth Cohort 1966, NFBC66 (I,II,IV)

The original cohort (Northern Finland Birth Cohort 1966, NFBC66) consisted of 12 058 live births from the two northernmost provinces of Finland, Oulu and Lapland, and covered 96% of the children born in that region between January 1 and December 31, 1966. In 1997, 8463 survivors still living in northern Finland or in the capital city area received a postal questionnaire and an invitation to attend clinical examinations; 6007 (50%) actually came to the examinations. The University of Oulu Ethics Committee approved the study and the participants provided written informed consent.

### 4.1.2 ALMA2-Study (III)

The study population consisted of adult women, living on farms around the city of Kuopio in eastern Finland. Study was conducted in 2002-2003. Subjects were drawn from an earlier cross-sectional study of mother-child pairs (Koskela et al., 2005) by inviting only those women living on farms and excluding subjects who had reported avoiding animal contact because of allergic diseases. Of the 169 invited women, 116 (69%) actually attended the examinations. The study plan was approved by the Research Ethics Committee, Hospital District of Northern Savo and informed written consent was obtained from every participant.

## 4.2 Questionnaire and variables

### 4.2.1 Assessment at age 31 years, NFBC66 (I,II,IV)

During follow-up at the age of 31 years, the participants underwent a clinical examination. At the 31-year follow-up, participants also filled in questionnaires, which included questions on health and lifestyle factors, socio-demographic factors, current exposure to respiratory hazards, current occupation, as well as allergic conditions. Allergic rhinitis, atopic eczema, doctor-diagnosed asthma and doctor-diagnosed chronic bronchitis/emphysema were based on self-report and were inquired separately for the last 12 months (labelled 'current' in the present analyses) and as ever having had the condition. Current allergic rhinitis, atopic eczema and asthma were analysed, but due to low numbers of current asthma (n=218) and atopic eczema (n=1059), asthma ever and atopic eczema ever were also analysed. Those who reported a parental history of allergic rhinitis, atopic

eczema and asthma were also identified. Moreover, there were questions about cat and dog ownership before the age of 7 years. Weight and height were measured and body mass index (BMI) was calculated as weight (kg) /height (m)<sup>2</sup>.

#### **4.2.2 Assessment of antenatal and childhood factors, NFBC66 (I,II,IV)**

Childhood information about parental professional farming, having farm animals (species and counts), maternal work with farm animals during pregnancy, place of residence, residential density, maternal education, maternal age, maternal BMI, smoking during pregnancy from the second month, mother's age of menarche and parity number, gestational age at birth, birth weight and height were collected by local midwives during pregnancy and/or immediately after delivery. Residential density was defined as the number of subjects in the household divided by the number of rooms in the household. Maternal work with farm animals during pregnancy was a subquestion for having farm animals in which subjects with farm animals during infancy were divided into three groups based on their mother's participation in work with farm animals during pregnancy (no maternal work with farm animals, maternal work with farm animals with assistance and maternal work with farm animals). Data obtained from the hospital discharge registry was used to identify subjects with pneumonia before the age of 7. Information about self-reported smoking at age 14 was collected from the questionnaire sent to participants and parents in 1980.

#### **4.2.3 ALMA2-Study (III)**

Based on self-administered questionnaires, information about current and childhood farm and home environment, such as contacts with cows and other cattle, hay barn visits during the past 12 months, pets in household and visible mould or smell of mould in household was also collected. The residential density was calculated by dividing the number of household occupants by the number of bedrooms, and three groups were formed: low (less than 1 persons/bedroom), medium (greater than or equal to 1 – less than 2 persons/bedroom) and high (greater than or equal to 2 persons/bedroom). Current exposure refers to the present habitual exposure. Subjects filled in the questionnaires a few days before blood sampling.

### **4.3 Skin prick tests (I, II, IV) and spirometry (II)**

During the follow-up conducted at age 31 years, participants underwent a skin prick tests to assess sensitivity to three of the most common allergens in Finland, i.e. cat, birch and timothy, and sensitivity to the house dust mite (*Dermatophagoides pteronyssinus*). Histamine dihydrochloride (10 mg/mL) and a diluent of the allergen extracts were used as positive and negative controls, respectively. Skin reactions to each allergen tested were recorded after 15 min as

the average of the maximum weal diameter and the diameter perpendicular to the maximum. Subjects with a weal reaction  $\geq 3$  mm to one or more of the four allergens tested were considered as atopic. Subjects with a positive reaction to the negative control or a negative reaction to histamine ( $< 3$  mm) were excluded.

During the follow-up examination conducted when subjects were aged 31 years, participants underwent spirometry. Lung function was assessed by forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV1) using a Vitalograph P-model spirometer (Vitalograph Ltd, Buckingham, UK) with a volumetric accuracy of 2% or 50 ml, whichever was greater. The spirometer was calibrated regularly using 1-litre precision syringe. The spirometric manoeuvre was performed three times but was repeated if the difference between two maximal readings for FEV1 or FVC was  $>4\%$ . The highest values of FEV1 and FVC were used.

#### 4.4 Cytokine analyses from peripheral blood (III)

Heparinized peripheral whole blood samples were collected, diluted 1:8 in culture media (RPMI 1640 with Glutamax supplemented with antibiotics/antimycotics [both from Gibco, Paisley, UK] and FCS Gold [PAA, Pasching, Austria] and stimulated for 24 h at  $+37^{\circ}\text{C}$ , 5%  $\text{CO}_2$  with the combination of phorbol myristate acetate (5 ng/ml) and ionomycin (1  $\mu\text{g/ml}$ ) (P/I), with staphylococcal enterotoxin B (0.1  $\mu\text{g/ml}$ ) (SEB) and with lipopolysaccharide (0.1  $\mu\text{g/ml}$ ) (LPS). Production of IFN- $\gamma$  and IL-4 was analysed from the supernatants by the ELISA method (R&D Systems, Minneapolis, MN). Methods used in cytokine analyses are similar as used in PASTURE birth cohort (von Mutius et al. 2006). These cytokines were selected to describe the Th1/Th2 balance. Complete cytokine data from analysed cytokines were available from 112 women. Cytokine concentrations were calculated both as picograms per milliliter and picograms per  $10^6$  leucocytes. The range of valid measurements was 5–1000 pg/ml for IFN- $\gamma$  and 8–2000 pg/ml for IL-4.

#### 4.5 Microbial analyses from house dust (III)

The study subjects were asked to collect two house dust samples, floor dust and vacuum cleaner dust bag dust. Methods used to collect dust samples have been generally used in other similar studies (von Mutius et al. 2006; Hyvärinen et al. 2006). The microbial analyses consisted of measurements of chemical markers of microbes (3-OH FAs, muramic acid and ergosterol) from dust bag dust and floor dust and cultivation of viable fungi and bacteria from dust bag dust (see more detailed methods in original publication III).

## 4.6 Statistical analyses

### 4.6.1 Northern Finland Birth Cohort 1966, NFBC66 (I,II,IV)

To analyse associations of farm-related factors during infancy with atopy and allergic diseases in adulthood, those 5509 subjects who had non-missing SPT data, and who had a positive reaction to histamine and a negative reaction to control were included to final analyses. Of these subjects, the maximum prevalence of missing data for disease outcomes was 1.5%, for farm characteristics collected during pregnancy was 1.3 % and for pet ownership collected at the 31-year follow-up was 4.5%. The highest prevalence of missing information for the confounders was 12.8 % (paternal allergy). Univariate analyses were carried out using the  $\chi^2$ -test and multivariate models were run using logistic regression. In order to select confounders for multivariate models, a list of potential confounders was identified a priori, i.e. sex, maternal age, maternal education, mother's smoking during pregnancy, maternal BMI, place of residence, residential density, current education, current BMI, current smoking, paternal asthma, paternal allergy, maternal asthma, maternal allergy, gestational age, mother's age of menarche, parity, birth height and weight. Current BMI, maternal BMI, birth weight and height were categorized in the analyses. In the multivariate models, missing data for each confounder listed above were classified as its own category. The final models included all variables that were associated with either atopy or doctor-diagnosed asthma at  $P < 0.2$  level. Only current smoking and birth weight were not significantly associated with either outcome and were therefore not adjusted for. All analyses were conducted with SPSS.

To analyse associations between farm-related factors during infancy and lung function in adulthood, those 5666 subjects for whom complete spirometry data was available and who were not pregnant were included to final analyses. Multivariate linear models were used to analyse associations between farm environment, potential confounders and lung function. Confounders for the multivariate models were selected a priori from a list of potential confounders. At the age of 31, potential confounders were height, weight, physical activity, education, smoking, exposure to cold, dust, solvents and tobacco smoke at work/or at home, paternal asthma, maternal asthma, paternal allergy and maternal allergy. Potential childhood confounders were height, weight and gestational age at birth, residential density, pneumonia before age of seven and smoking at the age of 14. Potential maternal confounders were mother's menarcheal age, parity number, age, education and smoking during pregnancy (after the second month of pregnancy). The final models included all variables, which were associated with outcome at  $p < 0.1$  level and which changed the estimate for the gender adjusted association between farm animal during infancy and outcome (FVC, FEV1) by



more than 10% (table 11). Models for FEV% (FEV1/FVC ratio) were adjusted with confounders selected for models for both FEV1 and FVC. With respect to included subjects, the maximum prevalence of missing data was as follows: for diseases 4.4 %, for farm characteristics collected during mother's pregnancy 0.8 %, for pet ownership collected at the 31-year follow-up 4.6 % and for data on current occupation was 2.9 %. In the statistical analyses, missing data for confounders were classified as its own category. The highest prevalence of missing information concerned allergic conditions in the father (12.8 %). All analyses were conducted with SPSS.

To explore whether analysing atopic or non-atopic asthma, as is typically done in epidemiologic studies, can lead to misleading associations those 5429 subjects with complete data from skin prick tests and asthma were included to final analyses. Asthmatics were divided into atopic and non-atopic asthmatics based on the presence or absence of atopy. The risks of atopy and different definitions of asthma were compared by calculating unadjusted relative risks (RR) and by estimating 2-sided p-values using the Chi-square test in Microsoft Excel. Multivariate logistic regression analyses were conducted with SPSS. The relative risks for atopic and non-atopic asthma were calculated in three different ways. Firstly, using the non-asthmatics as the comparison group, e.g. atopic asthmatics were compared to subjects without asthma. Secondly, by using those with neither asthma nor atopy as the comparison group. Thirdly, by comparing asthmatics to non-asthmatics separately in the atopics and non-atopics, i.e. doing an analysis stratified by atopy.

#### 4.6.2 ALMA2-study (III)

Complete cytokine data from analysed cytokines were available from 112 women. Uncorrected cytokine concentrations (pg/ml) and concentrations divided by the leukocyte count (pg/10<sup>6</sup> leukocyte) were well correlated both for IFN- $\gamma$  (LPS,  $r = 0.990$ ; SEB,  $r = 0.935$ ) and for IL-4 ( $r = 0.979$ ) (Spearman's nonparametric correlation). Therefore, only uncorrected cytokine concentrations (pg/ml) are presented. Maximum prevalence of missing data was 3.6% for farm-related factors. Due to non-normal distribution of the cytokine concentrations, Spearman's nonparametric correlations, Mann-Whitney U test and Kruskal-Wallis test were used to analyse associations between farm-related factors and peripheral blood cytokine production. All analyses were conducted using SPSS.



# 5 Results

## 5.1 Associations of farm environment during infancy with atopy, allergic diseases and lung function at age 31 (I,II)

A significantly decreased risk of atopy, allergic rhinitis, atopic eczema ever at age 31 and increased FEV1 and FVC at age 31 was seen in subjects who during their infancy had a parent who was a professional farmer (table 10, 11). Being born to a family having farm animal was associated with a decreased risk of atopy, doctor-diagnosed asthma ever, allergic rhinitis and atopic eczema ever (although some of the associations were not nominally significant) at age 31 (table 10) and with significantly increased FEV1 at age 31.

A reduced risk of atopy was particularly evident among those subjects whose mothers worked with farm animals during their pregnancy (for more detailed results see original publication I). Having a dog during childhood was significantly associated with a decreased risk of atopy (aOR 0.75 95% CI 0.66–0.85) and doctor-diagnosed asthma ever at age 31 (aOR 0.81 95% CI 0.65–1.00) and higher lung function (FEV1 36ml; 95% CI 10 to 61; FVC 37ml; 95% CI 8 to 67 ml) at age 31. Having a cat during childhood was associated with a decreased risk of atopy (aOR 0.68 95% CI 0.60–0.78) and allergic rhinitis (aOR 0.84 95% CI 0.74–0.96) at age 31. There was evidence of decreasing risk of atopy with increasing exposure to cows (for more detailed results see original publication I). Taken together, contact with an increasing number of animal species in childhood was dose-dependently and inversely associated with atopy at age 31 and similar inverse trend was also detected with allergic diseases (table 10). There was also suggestion for a dose-dependent association between number of animal species during childhood and increasing lung function at age 31, especially among women (table 11).

### 5.1.1 Exploring associations of farm environment during infancy with atopy, allergic diseases and lung function at age 31 (I,II)

Further adjustment for the family's professional farming while the subject was an infant had little effect on the strength or the significance of the associations between farm animal-related factors and atopy. When each animal species was considered as a separate exposure in the same model with potential confounders and socio-economic factors, only contact with cows (aOR 0.76 95% CI 0.61–0.93), cats (aOR 0.75 95% CI 0.65–0.86) and dogs (aOR 0.77 95% CI 0.68–0.88) remained significantly associated with the reduced risk of atopy.

To explore whether the associations between doctor-diagnosed asthma, allergic rhinitis and atopic eczema were being mediated via atopy, the above mentioned associations of having farm animals and the number of animal species were further adjusted for atopy. This clearly weakened all of the observed associations and no significant ORs were observed any longer with the number of animal species. Exposure to farm animals during infancy remained significantly associated only with atopic eczema ever (OR 0.81 95% CI 0.69–0.95), but not with doctor-diagnosed asthma ever (OR 0.83 95% CI 0.61–1.12). It was also explored whether atopy could modify the association between having farm animals and asthma, allergic rhinitis or atopic eczema. A significant interaction ( $P < 0.05$ ) was observed only for allergic rhinitis. However, the OR for exposure to farm animals was very similar in the atopics (OR 0.92 95% CI 0.68–1.25) and non-atopics (OR 1.05 95% CI 0.83–1.33).

To explore if the significant associations of farming environment during infancy with lung function at age 31 were being mediated via the protective effect of childhood farming on atopy or on respiratory diseases as such the models were further adjusted for atopy and respiratory diseases in separate models. The presence of atopy and doctor-diagnosed asthma reduced the size of the estimates rather strongly, but still only by about one fourth at the most. Adjustment for chronic bronchitis/emphysema also reduced the estimates, but in general to a lesser extent. When adjusting for atopy, for doctor diagnosed asthma and for chronic bronchitis/emphysema, the adjusted estimates for having a farming parent during infancy were 27 ml (95% CI -5 to 58 ml), 30 ml (95% CI -1 to 61 ml), 34 ml (95% CI 3 to 56 ml) for FEV1 and 36 ml (95% CI 0 to 72 ml), 34 ml (95% CI -1 to 69 ml), 36 ml (95% CI 1 to 71 ml) for FVC, respectively. The respective adjusted estimates for being born to a family with having farm animals during infancy were 23 ml (95% CI -7 to 53 ml), 23 ml (95% CI -6 to 52 ml) and 27 ml (95% CI -2 to 56 ml) for FEV1, respectively. Respective estimates for having a dog during childhood were 33 ml (95% CI 7 to 59 ml), 31 ml (95% CI 6 to 57 ml), 34 ml (95% CI 8 to 59 ml) for FEV1 and 36 ml (95% CI 5 to 66 ml), 34 ml (95% CI 4 to 63 ml) and 35 ml (95% CI 5 to 65 ml) for FVC, respectively.

Although associations between farming environment and higher lung function at age 31 were most evident among women, no statistically significant interactions ( $p < 0.05$ ) were observed. The smallest p-value was observed for the interaction between gender and being exposed to cats during childhood (FVC,  $p = 0.09$ ). The other p-values for the interaction between gender and having dogs in childhood, having farm animals or professional farming during infancy ranged between  $p = 0.18$  and  $p = 0.92$ . Also further adjustment for current farming occupation at age 31 had no impact on the size of the estimates of the observed significant associations with lung function.

**Table 10.** Adjusted associations of farm-related factors in infancy with atopy, asthma, allergic rhinitis and atopic eczema at age 31.

Farm-related factors in infancy	Study population		Atopy		Doctor diagnosed asthma ever		Allergic rhinitis*		Atopic eczema**	
	N	%	n	%	aOR*	95% CI	n	%	aOR	95% CI
Professional farming										
No	4247	77	1425	34	1		347	8	1	
Yes	1262	23	259	21	0.69	0.58 0.83	90	7	0.96	0.72 1.29
Family had farm animals										
No	3811	70	1323	35	1		327	9	1	
Yes	1653	30	344	21	0.67	0.56 0.80	103	6	0.74	0.55 1.00
Number of animal species#										
0	1428	28	565	40	1		131	9	1	
1	1500	30	527	35	0.88	0.75 1.03	123	8	0.85	0.65 1.11
2	1017	20	256	25	0.59	0.49 0.72	83	8	0.81	0.59 1.11
3	645	13	123	19	0.45	0.35 0.59	41	6	0.61	0.40 0.95
≥ 4	472	9	83	18	0.43	0.32 0.58	26	6	0.56	0.34 0.92

aOR: Adjusted for sex, maternal age, maternal education, smoking during pregnancy, maternal BMI, place of residence, residential density, education at age 31, BMI at age 31, paternal asthma,

paternal allergy, maternal asthma, maternal allergy, gestational age, mother's age of menarche, parity and birth height

Atopy: Subjects with a wheal reaction ≥ 3 mm to one or more of the four allergens tested were considered to be atopic

# Before age of seven; \*Self-reported in past 12 months; \*\*Self-reported ever; # Includes cows, pigs, sheep, poultry, miniks, cats and dogs.

**Table 11.** Associations between farm-related factors during infancy and lung function at age 31.

Farm-related factors in infancy	N	FEV1 difference (ml)		FVC difference (ml)	
		crude	adj. (95% CI)	crude	adj. (95% CI)
Professional farming					
No	4367	0	0	0	0
Yes	1299	44	36 (6 to 67)	42	40 (5 to 75)
Family had farm animals					
No	3930	0	0	0	0
Yes	1692	39	32 (3 to 61)	14	11 (-22 to 43)
Number of animal species# <sup>α</sup>					
0	1476	0	0	0	0
1	1539	39	39 (6 to 73)	41	41 (2 to 79)
2	1046	53	56 (18 to 94)	32	30 (-14 to 74)
3	663	67	65 (20 to 109)	54	51 (0 to 103)
≥ 4	485	55	44 (-6 to 94)	48	43 (-14 to 100)

<sup>α</sup> Before age of seven; # includes cows, pigs, sheep, poultry, minks, cats and dogs.

adj.: All the models were adjusted for gender, height at age 31, weight at age 31, smoking at age 31, education at age 31, exposure to the solvents and tobacco smoke at home at age 31, physical Activity age 31, birth height and weight, maternal education. In addition, model for FEV1 was adjusted for smoking at age 14, paternal asthma and maternal age and the model for FVC for residential density.

Crude: adjusted for gender and height at age 31

## 5.2 Cytokine production in farming women (III)

Current farm-related factors, such as work with cows, hay barn visits or pet ownership had only minor effects on stimulated peripheral blood cytokine production in the farming women studied here. Similarly, living on an operational farm during childhood or living in a household with pets during childhood had no significant associations on the stimulated cytokine responses. Subjects reporting the presence of mould (n=18) in their household had significantly higher concentrations of LPS-stimulated IFN- $\gamma$  (median [pg/ml] 44 vs. 18; P-value 0.03) production in their peripheral blood.

The measured microbial exposure had little effect on the stimulated cytokine production in the peripheral blood. The only statistically significant correlations were observed between *Cladosporium* and P/I-stimulated IL-4 ( $r = 0.26$ ,  $p = 0.02$ ) and between ergosterol (dust bag) and SEB-stimulated IFN- $\gamma$  ( $r = -0.26$ ,  $p = 0.02$ ). All of the other correlations between measured microbial exposures and cytokine production had p-values above 0.05. For more detailed results see original publication III.

## 5.3 Analysing atopic and non-atopic asthma (IV)

Having a cat during childhood was significantly inversely associated (relative risk, RR 0.67,  $P < 0.001$ ) with atopy at the age of 31 year. There was no significant association with asthma (RR 0.91,  $P = 0.3$ ), but a significant inverse association with atopic asthma (RR 0.72,  $P < 0.01$ ) and a positive association with non-atopic asthma (RR 1.36,  $P < 0.05$ ), when non-asthmatic subjects were used as the comparison group. These results remained essentially unchanged, if atopic and non-atopic asthma were analysed in the same way as asthma and atopy, i.e. calculating the risk in the whole study population, as the number of asthmatics was so small (data not shown). When using those with neither asthma nor atopy as the comparison group, the relative risk for atopic asthma was even further away from one (RR 0.62,  $P < 0.001$ ) than the original result (RR 0.72). In contrast, the relative risk for non-atopic asthma was 1.16 ( $P = 0.3$ ), which is the same as the estimate for stratified analysis among non-atopics (RR 1.16), i.e. the analyses are exactly the same.

When the analysis were stratified according the presence atopy, i.e. the analysis were conducted separately for atopics and non-atopics, no association was found between cat ownership and asthma in atopics (RR 1.07,  $P = 0.6$ ) or non-atopics (RR 1.16,  $P = 0.3$ ) was found. This indicates that atopy did not modify the association of cat ownership with asthma and that the above apparently very strong associations with atopic (RR 0.72,  $P < 0.01$ ) and non-atopic asthma (RR 1.36,  $P < 0.05$ ) were misleading. When adjusting for atopy using multivariate

logistic regression, the weak protective association between cat ownership and asthma also disappeared; the odds ratio between cat ownership and asthma became 0.86 (95% CI 0.70–1.05) without adjustment for atopy but 1.06 (95% CI 0.85–1.30) after adjustment for atopy. Both models are adjusted also for sex, parental allergy, and current education.



# 6 Discussion

## 6.1 Does living on a farm in childhood protect from allergic diseases?

This doctoral thesis shows that being born to a family possessing farm animals was associated with a decreased risk of atopy when the subjects were aged 31. The results remained independent of the family's professional farming, socio-economic differences and other potential confounders between subjects. Previous studies, mainly conducted with cross-sectional designs, have suggested that living in a farming environment during early childhood may protect against atopy and allergic diseases (see 2.2-2.3). However, this is the first prospective longitudinal study investigating associations of farm environment, especially farm animal contact, in early childhood with atopy and allergic diseases in adulthood.

The present study also suggests a strong dose-dependent and inverse association between contact both with an increasing number of animal species and with the total number of cows during infancy and atopy in adulthood. A similar proposal has been reported in a few cross-sectional studies (Remes et al 2003, Riedler et al. 2001). Dose-dependent associations point to some plausible biological mechanism behind the protective effect. The present observations are compatible with the hypothesis that it is the quality and quantity of environmental microbial exposure in farming environment that may govern the development of the immune system during infancy and in that way confer prevention from atopy and allergic diseases (von Mutius et al. 2010).

Similarly but somewhat weaker, farm-related factors in infancy were associated with a decreased risk of allergic diseases at age 31, as with atopy. However, most of the associations were explained by atopy, although e.g. of asthma little more than half is attributable to atopy (Pearce et al. 1999). These observations are compatible with the hypothesis that microbial exposure in the farming environment during infancy may influence the risk of developing atopy (von Mutius et al. 2010). Further, it is atopy that may be driving the observed associations of allergic diseases.

Cross-sectional studies have suggested that prenatal contact with farm animals may also reduce the risk of atopy (Riedler et al. 2001, Ege et al. 2006). Based on the reviewed literature which contains mainly data from cross-sectional studies,

the effect of the farming environment is probably most evident at the time of immune system priming, particularly during the first years of life or even at the time of fetal development. Interestingly, this study suggests that a decreased risk of atopy in adulthood is particularly evident among subjects whose mothers were working with farm animals during pregnancy.

Although pet ownership is not directly associated with a farming environment, in Finland pets are more common in farming families as compared to non-farming families (Remes et al. 2003). Present study suggests that having a cat or a dog in the household during childhood was associated with the decreased risk of atopy at the age of 31 independently from contact with cows. Furthermore, there was tendency that childhood pet ownership was associated with a reduced risk of the studied allergic diseases in adulthood. Cat ownership was statistically significantly associated with a reduced risk of allergic rhinitis at age 31 and dog ownership was statistically significantly associated with reduced risk of doctor-diagnosed asthma ever at age 31. These findings are in line with evidence from previous studies (see 2.3.3).

## 6.2 Living on a farm in early childhood and lung function at adult age

This is the first prospective birth cohort study to describe the associations between farming environment during infancy and lung function at adult age. The results suggest that farming environment during infancy is associated with higher FEV1 and FVC at age 31 although no associations were observed with FEV%. These findings suggest that living on a farm in early childhood may have a positive impact on lung function in adulthood.

Respiratory diseases are well known occupational health problem among farmers (Linaker et al.; 2002). Previous studies have revealed that farming as an occupation seems to be a risk factor for lung function impairment (see 2.5.2). The risk seems to be associated more strongly with working with farm animals i.e. dairy and pig farming and lung function seems to decline as the exposure continues. However, the effect of childhood farming environment on lung function has been investigated in only a few cross-sectional studies with most studies claiming that living on a farm in childhood has no effect on lung function on childhood or adulthood (see 2.5.1). Furthermore, there are no prospective longitudinal studies examining associations between the farming environment during childhood and adult lung function.

In contrast, the quality and quantity of environmental microbial exposure, such as exposure to bacterial endotoxin (Gram-negative bacterial cell wall component),

in the farming environment may affect the development of the immune system during childhood and in that way, confer protection against asthma. (Von Mutius et al. 2010). On the other hand, a high exposure to endotoxin may also irritate the airways and be a source of airway inflammation and obstruction not only in the occupational setting (Radon 2006a), but also during early childhood (Mendy et al. 2011). A meta-analysis revealed that current endotoxin exposure protected from asthma when children were of school-age, but early childhood exposure increased the risk of wheezing during infancy (Mendy et al. 2011). Therefore, living in farm environment during early childhood may reduce the risk of asthma, whereas exposure during adulthood seems to be a risk factor for occupational respiratory diseases.

In the present study living in a farming environment during infancy was associated with higher FEV1 and FVC but not with FEV%. These spirometric findings point to a restrictive type lung function impairment among subjects without contact to the farming environment during infancy. This is an unexpected finding, since the farming environment is mainly associated with obstructive disorders. However, as our lung function measurements consisted of FEV1 and FVC, the type of the lung function impairment cannot be definitely identified. One potential mechanism for the present restrictive type findings may be obstruction in small airways caused by patchy collapse in early exhalation. In such case, FEV1 and FVC will be decreased and FEV% is normal (Pellegrino et al. 2005). However, the possible contribution of airway obstruction on the suspected restrictive-type impairment would require a bronchodilator test. Confirmation of a true restrictive abnormality would require total lung capacity measurement either with a body plethysmograph or the gas dilution methods (Flesch et al. 2012).

The findings in this thesis, that the farming environment during early childhood is associated with reduced risk of atopy and allergic diseases at the adult age, are potential explanations for the present observations. However, it was decided to conduct separate analyses and to construct models which were further adjusted for atopy or doctor-diagnosed asthma. In these evaluations the size of the estimates for the association between early childhood farming environment and current lung function was reduced by only about one fourth at the most. This suggests that although atopy probably partly mediates the effect on improved lung function also other pathways are likely to be involved. It was also decided to adjust the analyses for doctor diagnosed chronic bronchitis or emphysema. This also reduced the size of the estimates but to a slightly lesser extent. This suggests that being exposed to farming environment early in life may not only prevent from development of chronic respiratory disease, but may also improve lung function in all exposed subjects.

In general, there may be gender differences in the respiratory responses to different environmental exposures. A recent meta-analysis detected a higher risk of asthma in women exposed to organic and inorganic dusts as compared to men. In contrast, exposure to organic dust has been linked with lower lung function in men than in women (Dimich-Ward et al. 2012). In the present study, the observed associations were more evident among women, but this difference between genders did not quite reach statistical significance. These gender-related differences may be due to differences in biology and in exposure patterns (Clougherty 2010).

Differences in physical activity could also explain the observed differences in lung function. Physical activity is known to be associated with higher lung function, as measured with FEV1 in adulthood, in both men and women of all ages (Nystad et al. 2006). In the present study, the analyses were adjusted for current, but not for childhood physical activity. It is possible that children living on farms during childhood have higher physical activities, as they may participate in more physically demanding tasks on farm, as compared to their peers living in non-farming environments. Unfortunately, there is lack of information about the physical activity levels during subject's childhood and the effect of childhood physical activity on the lung function at age 31 could not be estimated.

As the differences in current (Koenig 2001) or birth (Canoy et al. 2007) weight of the subjects could offer a simple explanation for the current findings, the models were adjusted for both current and birth weight. In the sensitivity analysis, the models were also adjusted for body mass index at age 31 instead of weight at age 31, but the results remained unchanged. Therefore the associations found between living in a farming environment during infancy and lung function at adulthood are unlikely to be explained by obesity.

Clinical significance of these relatively small mean changes in lung function remains unclear. However, it has been shown that air pollution associated relatively small changes in mean lung function may also reflect in prevalence of individuals with clinically important changes (Raizenne et al. 1996). Therefore, small mean changes in lung function at age 31 associated with farm environment during early childhood may reflect clinically relevant changes in some individuals.

### 6.3 Immune system modulation in adulthood

There is evidence from previous studies that farm-related exposures during early childhood or during mother's pregnancy may affect the development of the immune system in childhood (see 2.6.1). However, the capacity of farm-related

exposures to affect immune responses in adulthood is less clear (see 2.6.2). As far as is known, this thesis is the first study to investigate association between farm-related factors, especially those associated with elevated microbial exposure, and stimulated production of IFN- $\gamma$  and IL-4 in peripheral blood samples of adult female farmers. Only a few significant associations were found, which suggests that environmental modulation of the immune system in adulthood is relatively weak. This is in line with evidence from reviewed literature (see 2.6.2)

The present study, gathered comprehensive data on microbial agents (chemical markers for Gram-negative and Gram-positive bacteria and fungal biomass, viable fungi, viable bacteria). Only the dust bag concentration of ergosterol as a marker of fungal biomass displayed a significant negative correlation with SEB-induced IFN- $\gamma$  production and the concentration of *Cladosporium*, which is a common outdoor and indoor fungus especially in the farm environment (Kotimaa et al., 1991), had a positive correlation with P/I-induced IL-4 production. These findings suggest that an intense fungal exposure may be associated with Th2-skewed responses and therefore could be a potential risk factor for allergic diseases. In contrast, Beijer et al. (2003) has reported increased ratio of IFN- $\gamma$  to IL-4 in adult non-atopic subjects with high exposure to airborne (1 $\rightarrow$ 3)-d-glucan, an indicator of mould. Furthermore, in their recent study Lappalainen et al. (2008) detected no associations between ergosterol in the house dust and stimulated IFN- $\gamma$  production in peripheral blood samples (Lappalainen et al. 2008).

In this study, current farm-related factors associated with elevated microbial exposure (work with cows, hay barn visits, household pets), exerted only minor effects on stimulated IFN- $\gamma$  production in peripheral blood of adult females, in contrast to previous studies conducted during early childhood (Pfefferle et al., 2010; Roponen et al., 2005, Perkin et al. 2006). Here, self-reported mould in the household was associated with a higher production of LPS-induced IFN- $\gamma$ . However, the effects of mould exposure in immune responses clearly need to be studied in future, since this finding is based on self-reported presence of mould in the household and it was not supported by any associations between cytokines and objective measurements of microbial agents in the house dust. Similarly to current farm-related factors, farm-related factors experienced during childhood exhibited no significant association with stimulated cytokine production during adulthood.

## 6.4 Methodological issues

### 6.4.1 Analysing atopic- and non-atopic asthma

This study illustrates that analysing atopic or non-atopic asthma as is typically done, i.e. using all other participants or non-atopic non-asthmatics as the

comparison group, can lead to misleading associations. Therefore, asthma and atopy are best analysed as separate outcomes. If atopic and non-atopic asthma are analysed separately, valid results can only be obtained by using the correct comparison group, i.e. by comparing atopic asthmatics to atopic non-asthmatics and by comparing non-atopic asthmatics to non-atopic non-asthmatics. This is the equivalent to conducting a stratified analysis, where the data is stratified by atopy.

Asthma is not a single disease entity as is general known, but defining the phenotypes is difficult (Wenzel 2006; Global Initiative of Asthma 2012). In many studies, especially large epidemiological studies, the main data available on the asthma phenotype is data about asthma and atopy. In this situation, asthmatics are typically divided into atopic and non-atopic asthmatics only based on atopy. However, it is well known that atopic and non-atopic asthma are not well separated disease entities and they should therefore not be used (Lötvall et al. 2011; Global Initiative of Asthma 2012). In such a disease definition, there is severe disease misclassification, as the presence or non-presence of atopy is a poor marker for the allergic nature of asthma (Global Initiative of Asthma 2012; Pearce et al. 1999).

Several of the currently known risk factors of atopy and allergic diseases are more strongly associated with atopy than with asthma. This is true for several factors related to the hygiene hypothesis, such as infections and environmental microbial exposure (Eder et al. 2006). In this situation, it is likely that associations with atopy drive the observed associations with atopic and non-atopic asthma. Therefore, studies often find much stronger and statistically significant associations with atopic asthma than for asthma and atopy analysed separately. This was the case in the present thesis for cat ownership, where the associations were significant both for atopic asthma (protective) and non-atopic asthma (risk factor), but no associations were observed with asthma. The awkward inverse associations with non-atopic asthma only emphasize the problems of these comparisons.

In the literature review in this thesis, there are a few studies reporting associations of farming environment during childhood with atopic and/or non-atopic asthma. Braun-Fahrlander et al. (2002) described an association of farming environment in childhood and endotoxin exposure in homes with atopic/non-atopic asthma without stratification. In prospective birth-cohort study by Kiechl-Kohlendorfer et al. (2007) atopic-asthma was the only outcome when associations with farming environment during infancy were analysed. Similar analyses with potentially problematic comparison groups when analysing atopic- and non-atopic asthma have been published (e.g. Radon et al. 2004b)

In this study, when the association of farming environment during infancy with atopy and asthma in adulthood was analysed, this was estimated via separate endpoints. To explore whether the associations with asthma are mediated via atopy, the associations needed to be adjusted for atopy. Furthermore, to explore whether atopy modifies the associations between having farm animals and asthma, a stratified analyses was performed. In these analyses no significant interactions were observed ( $P < 0.05$ ), suggesting that there is no difference between atopics and non-atopics in the association between having farm animals during infancy and risk of asthma.

#### 6.4.2 Strengths and weaknesses

The size of the study population and the study design of NFBC66 were major strengths of this thesis. The NFBC66 consisted of 12 058 live births and in the 1997 follow-up study 6007 (50%) subjects attended the examinations. In this large population-based birth cohort, it was possible to collect detailed information about subjects' farming environment during infancy without possible recall bias. The present study appears to be the first prospective birth cohort study reporting associations of the farming environment during infancy, especially farm animal contact, with atopy, allergic diseases and lung function at adult age.

The other major strength in NFBC66 was the heterogeneous study population. When the farm environment information was collected in 1966, Finland was still in many respects an agricultural society. At that time, farm animal ownership was not restricted to subjects whose parents were professional farmers. In the multivariate analysis, the effect of having farm animals during infancy on atopy were adjusted for both place of residence and family's professional farming. This weakened the effect of having farm animals and may in fact have led to overadjustment, but the adjustment did not abolish the effect. Hence, disentangling between the effect of having farm animals in infancy and being born to a family practicing professional farming was possible.

In the NFBC66 study, even with its strong prospective birth cohort design, there are a few weaknesses. The healthy worker effect is always a concern (i.e. selection bias). There is the possibility that some parents of the children selected other occupations or otherwise avoided the farm environment since they experienced allergic or respiratory diseases, which could lead to differences in the genetic predispositions between those living and those not living on a farm in infancy. This cannot be ruled out even in this prospective birth cohort. The potential effect is only partly controlled by adjusting for parental allergies and asthma. Furthermore, there was no suggestion that there was a healthy worker

effect in adulthood, as there was no change in the observed estimates in the lung function analyses after adjustment for current farming at age 31. Therefore, the healthy worker effect is an unlikely explanation for the present findings.

One other weakness was that NFBC66 study lacked information on farm milk consumption during childhood and data on childhood pet ownership was only retrospective. Selection among subjects not participating in the 31-year follow-up might also misrepresent the observed associations since 50% of subjects attended to follow-up and no drop-out analysis were performed. However, it is unlikely that there would be major differences among subjects with farm childhood compared non-farming childhood with respect to participation rate.

Because the present study lacked information on farm animal contact after infancy as well as intermediate assessments of allergic diseases and lung function, it was not possible to conduct detailed assessments of the effect of being in contact with farm animals at different time-points. Therefore, the effects of prenatal exposure, early childhood exposure and current exposure cannot be definitely differentiated.

The ALMA2 study in this thesis had a cross-sectional design and it consisted of adult women living on farms around the city of Kuopio in eastern Finland. Of the 169 invited women, 116 (69%) attended. In contrast to many previous similar studies examining stimulated peripheral blood cytokine production, the present study was population-based and had a fairly large sample size.

The weakness of the ALMA2 study was that the study data provided only limited information on the function of the immune system in adult females, since only two cytokines were measured. IFN- $\gamma$  and IL-4 were selected to describe the Th1/Th2 balance. Furthermore, the results may not be directly generalized to male gender (Casimir et al. 2013). It is also possible that there were weak associations between environmental factors and immune responses that could not be detected with the current design and sample size. Another limitation of the study was the lack of personal exposure measurements or air samplings in stables and barns. In the case of farming adults, the most intense microbial exposure probably occurs while doing actual farm work, i.e., measurements of the microbial concentrations in house dust would not completely reflect the personal exposure levels of the study subjects. However, previously concentrations of microbes and microbial markers in house dust have been shown to differ between farmers and non-farmers (Braun-Fahrlander et al., 2002; Kärkkäinen et al., 2010), which suggests that house dust measurements at least partly describe the quantity and quality of exposures also outside the home.



# 7 Conclusions and future directions

Based on the evidence presented in this thesis, the following conclusions and directions for future studies can be drawn:

1. Contact with farm animals in infancy is associated with a reduced risk of atopy, doctor-diagnosed asthma, allergic rhinitis and atopic eczema at age 31. Further studies should be aimed to determine the protective factors beyond farm animal contact and the associated gene-environment interactions.

2. Exposure to a farming environment during infancy was associated with higher FVC and FEV1, but not FEV% when subjects were aged 31. This suggests that farming environment in early childhood may have a positive impact on lung function in adulthood, but this will need to be confirmed in future studies.

3. It seemed that the capacity of the studied environmental factors to influence the studied cytokines was relatively weak in adulthood. Therefore, the potential of microbial exposures and other farm-associated factors to influence immune responsiveness remain to be identified in future studies.

4. In epidemiologic studies, if only data on asthma and atopy are available, the asthma and atopy are best analysed as separate outcomes. If atopic and non-atopic asthma are analysed as additional end points, the valid results can be obtained by stratifying the analysis by atopy.

5. Both epidemiological and immunological studies presented in this thesis support the hypothesis that the protective effect of farm environment, especially farm animal contact, against development of atopy and allergic diseases is most evident for exposure occurring during the first years of life and/or prenatally. However, the intriguing possibility for protective effect of adult exposures requires further study.



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